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(54) Title: CO-ADMINISTRATION OF A POLYSACCHARIDE WITH A CHEMOTHERAPEUTIC AGENT FOR THE TREATMENT OF CANCER

(57) Abstract: Methods and compositions for treating cancer with a formulation are provided in which a polysaccharide, galactomannan is coadministered with a chemotherapeutic agent to a subject to reduce toxicity and/or to enhance efficacy of the agent for the subject.

Co-administration of a Polysaccharide with a Chemotherapeutic Agent for the Treatment of Cancer

Technical Field and Background Art

The present invention relates to administration of a toxic agent to a subject with cancer, in a formulation in which toxicity is substantially reduced and/or therapeutic efficacy enhanced. The most widely used methods to treat cancer are surgery, radiation and chemotherapy. Cancer patients often receive a combination of these treatments and about half of all patients receive chemotherapy. Unfortunately, chemotherapeutic agents have significant limitations relating to their toxic effect on the patient and the efficacy of a particular dosage to target and kill tumor cells.

Most chemotherapy agents kill cancer cells by disrupting the cell division process. Cells are killed once they begin to undergo division and replication. Although these agents are effective for treating cancer cells which generally grow rapidly through unregulated cell division, they also kill healthy non-cancerous cells as they undergo ordinary cell division. This toxic effect is particularly apparent in fast-growing normal cells, such as bone marrow cells, those in the digestive tract, hair follicles, and reproductive cells. Because chemotherapy harms healthy tissue, the effectiveness of the drug is limited by dosage levels and treatment frequency which should not exceed tolerance levels for non-cancerous cells. Moreover, the chemotherapy regimen often dramatically diminishes the quality of a patient's life through its physical and emotional side effects. Without the ability to target the drug exclusively to cancerous tissue, chemotherapy dosages must be kept within a range that healthy tissue can tolerate, thus reducing the optimal effectiveness of chemotherapy on diseased tissue. If the toxicity of chemotherapeutic agents could be reduced, the clinician would be able to increase the dosage of drug without causing unacceptable side effects. Increasing efficacy of a drug can be translated into decreasing of the dosage of the drug, which again minimizes the harmful effects on the patient while offering maximum benefit. Decreasing dosage by increasing efficacy of a chemotherapeutic drug together with a reduction in toxic effects

would lead to improvement of the patient's quality of life through controlling the tumor and through harmful side effects.

Summary

In a first embodiment of the invention there is provided a method for treating a cancer in a subject, that includes obtaining a mixture of galactomannan polysaccharide and an effective dose of a chemotherapeutic agent in a pharmaceutically acceptable formulation; and administering the formulation to the subject so as to treat the cancer.

In additional embodiments, the mixture contains an amount of galactomannan and the therapeutic agent in a ratio suitable for reducing a toxic effect in the subject, the toxic effect resulting from administration of a cancer-reducing amount of the chemotherapeutic agent absent galactomannan, the mixture optionally enhancing efficacy of chemotherapeutic effect for treating the cancer.

In further embodiments of the method, the molecular weight of the galactomannan is in the range of 20,000- 600,000 D, for example the galactomannan has a molecular weight in the range of 90,000 to 415,000 D or 40,000-200,000 D, for example, the galactomannan has an average molecular weight of 83,000 D or 215,000 D. The galactomannan may be a derivative of an isolate from *Gleditsia triacanthos* or from *Medicago falcate* or from *Cyamopsis tetragonoloba.*

In further embodiments, the galactomannan may be β-1→4-D-galactomannan and include a ratio of galactose to mannose where mannose is in the range of 1.0-3.0 and galactose is in the range of 0.5-1.5. Alternatively, the galactomannan includes a ratio of 2.6 mannose to 1.5 galactose.

In further embodiments, the galactomannan includes a ratio of 2.2 mannose to 0.9 galactose. Alternatively, the galactomannan may include a ratio of 1.13 mannose to 1 galactose. Alternatively, the galactomannan includes a ratio of 2.2 mannose to 1 galactose.

In further embodiments, the galactomannan and the chemotherapeutic agent are present in the mixture in a ratio of 0.1:1w/w to 10:1w/w.

In further embodiments, the mixture has a reduced toxicity of greater than 50% compared with the same dose of the agent absent galactomannan. For example, the mixture has a reduced toxicity of greater than 80% compared with the same dose of the agent absent galactomannan.

In embodiments of the invention, the chemotherapeutic agent is 5-FU. The cancer may be any of chronic leukemia, breast cancer, sarcoma, ovarian carcinoma, rectal cancer, throat cancer, melanoma, colon cancer, bladder cancer, lung cancer, mammary adenocarcinoma, gastrointestinal cancer, stomach cancer, prostate cancer, pancreatic cancer, or Kaposi's sarcoma. For example, the cancer may be any of breast cancer, colon cancer, or pancreatic cancer. Any of the above is applicable to a human subject.

In an embodiment of the invention, a pharmaceutical formulation is provided that includes a mixture of galactomannan polysaccharide and an effective dose for treating cancer of a chemotherapeutic agent in a pharmaceutically acceptable formulation. The mixture in the formulation may contain an amount of galactomannan and the therapeutic agent in a ratio suitable for reducing a toxic effect in the subject, the toxic effect resulting from administration of a cancer treating amount of chemotherapeutic agent absent galactomannan. Furthermore, the mixture may contain an amount of galactomannan and the therapeutic agent in a ratio suitable for enhancing efficacy of chemotherapeutic effect for treating the cancer.

In an embodiment of the invention, a method is provided where the mixture contains an amount of galactomannan and the therapeutic agent in a ratio suitable for enhancing efficacy of chemotherapeutic effect for treating the cancer. For any of the above, the formulation may be in a powder form.or in a liquid form.

In an embodiment of the invention, a method is provided for obtaining a mixture of galactomannan polysaccharide and an effective dose of a chemotherapeutic agent formulated so that the chemotherapeutic agent has reduced toxicity in the presence of the galactomannan, the formulation being suitable for parenteral administration to the subject; and administering the formulation to the subject so as to treat the cancer.

In an embodiment of the invention, a method is provided for treating cancer in a subject, that includes obtaining an effective dose of a mixture of galactomannan polysaccharide and an effective dose of a chemotherapeutic agent formulated so that the chemotherapeutic agent has enhanced therapeutic efficacy in the presence of the galactomannan, the formulation being suitable for parenteral administration to the subject; and administering the formulation to the subject so as to treat the cancer. A method according to the above claims wherein the chemotherapeutic agent is adriamycin or 5 fluorouracil. A method according to any of the above embodiments wherein the enhanced therapeutic effect is a synergistic effect.

Detailed Description of Embodiments

As used in this description and the accompanying claims, the following terms shall have the meanings indicated, unless the context otherwise requires.

"Subject" refers to a mammal including a human in need of therapy for, or susceptible to, a condition or its sequelae. The subject may include dogs, cats, pigs, cows, sheep, goats, horses, rats, and mice and humans. The term "subject" does not exclude an individual that is normal in all respects.

"Patient" refers to a human subject who has presented at a clinical setting with a particular symptom or symptoms suggesting the need for treatment.

"Polysaccharide" refers to polymers comprised primarily of monomers of one or more sugars and substituted sugars. When isolated from nature, polysaccharide preparations comprise molecules that are commonly heterogeneous in molecular weight.

"Efficacy" for a toxic therapeutic agent refers to the relationship between a minimum effective dose and an extent of toxic side effects. Efficacy of an agent is increased if a therapeutic end point can be achieved by administration of a lower dose or a shorter dosage regimen. If toxicity can be decreased, a therapeutic agent can be administered on a longer dosage regimen or even chronically with greater patient compliance and improved quality of life. Further, decreased toxicity of an agent enables the practitioner to increase the dosage to achieve the therapeutic endpoint sooner, or to achieve a higher therapeutic endpoint. "Efficacy" for a non-toxic therapeutic agent relates to improved therapeutic effect for treating a condition

"Pharmaceutically acceptable carrier" refers to any and all solvents, dispersion media, e.g., human albumin or cross-linked gelatin polypeptides, coatings, antibacterial and antifungal agents, isotonic, e.g., sodium chloride or sodium glutamate, and absorption delaying agents, and the like that are physiologically compatible. The use of such media and agents for pharmaceutically active substances is well known in the art. Preferably, the carrier is suitable for oral, intravenous, intramuscular, subcutaneous, parenteral, spinal or epidural administration (e.g., by injection or infusion). Depending on the route of administration, the active compound can be coated in a material to protect the compound from the action of acids and other natural conditions that can inactivate the compound.

"Parenteral administration" includes administration by bolus injection or infusion, as well as administration by intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal,

subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

"Toxic" refers to any adverse effect caused by an agent when administered to a subject.

"Tumor regression" was scored (excluding nonspecific deaths) as "partial" (less than 50 percent of its size at the beginning of treatment), or "complete" (tumor becomes unpalpable).

"Duration of regression" refers to the interval during which a tumor classified as a partial or complete regression continues to be below 50 percent of its size at first treatment.

"Evaluation size" refers to the tumor mass selected at one or two mass doubling beginning with the initial tumor size at the start of treatment.

"Time required for tumor mass doubling" is the time to reach the evaluation size; it is used in the calculations of the overall delay in the growth of the median tumor [(T-C)/C x 100 %], where T-C (days) is the difference in the median of times postimplant for tumors of the treated (T) groups to attain an evaluation size compared to the median of the control (C) group. The T-C value is measured excluding nonspecific deaths, and any other animal that dies whose tumor failed to attain the evaluation size.

We provide a method for treating a subject with a therapeutic agent that minimizes the toxic side effects of the therapeutic agent and may additionally enhance its therapeutic efficacy. The method requires the co-administration of the agent with a polysaccharide.

Although the examples provided herein describe the beneficial effects of galactomannans, we do not exclude the possibility that other polysaccharides may have a similar effect. The observed reduction in toxicity of a toxic therapeutic agent makes it possible to administer a greater dose without an increase in adverse side effects associated with treatment. The administration of increased dosages of a therapeutic agent having toxic side effects may be beneficial for treatment of a number of diseases including cancer, where the toxic side effects of traditional cytotoxic agents have limited their use.

In addition to reducing toxicity, efficacy of the therapeutic effect may be enhanced by administering a therapeutic agent with a galactomannan. The increase in efficacy may arise from a synergistic effect between the galactomannan and the therapeutic agent mixture.

Both the polysaccharide and the agent may separately be formulated, in a dry form for example as a powder, or in a liquid form. In a preferred embodiment, the polysaccharide and therapeutic agent are mixed prior to administration. The mixture may be in the form of a liquid, a powder or an aerosol.

The dosage regimens for established chemotherapeutic agents with known toxic side effects has been established and are described in the Physician's Desk Reference. For example, a description of adriamycin can be found in the Physician's Desk Reference 48th Edition (1994) pp. 459-461 and for 5-fluorouracil (5-FU) on pages 1924-1925, these descriptions being incorporated by reference. The co-administration of polysaccharide with a therapeutic agent may utilize but is not limited to the dosage regimen and route of administration already established and approved for the therapeutic agent, the difference being the inclusion of the polysaccharide. For example, a single bolus can be administered, several divided doses can be administered over a period of time, or a dose can be proportionally reduced and administered over a time period by infusion, or can be increased, as indicated by the exigencies of the therapeutic situation. The dosage unit will be a mixture of polysaccharide with therapeutic agent. However, we do not exclude the possibility that the polysaccharide and therapeutic agent could be administered sequentially as distinct formulations.

The formulation of the mixture may be derived from the standard formulation of the therapeutic agent to which the polysaccharide is added in a compatible solvent or as a powder. For example, the chemotherapeutic agent 5-FU is commonly formulated in an aqueous solution with excipients. In Example 1, aqueous galactomannan was added to the aqueous 5-FU to provide the formulation that was administered to the subject.

Pharmaceutically acceptable carriers are commonly added in typical drug formulations. For example in oral formulations, hydroxypropyl cellulose, colloidal silicon dioxide, magnesium carbonate, methacrylic acid copolymer, starch, talc, sugar sphere, sucrose, polyethylene glycol, polysorbate 80, and titanium dioxide: croscarmellose sodium, edible inks, gelatin, lactose monohydrate, magnesium stearate, povidone, sodium lauryl sulfate. carnuba wax, crospovidone, hydroxypropyl methylcellulose, lactose, microcrystalline cellulose, and other ingredients may be used. In addition, galactomannan has been used as a carrier for oral delivery of agents, which are in a non-liquid form. (US Patent Nos: 4,447,337; 5,128,143; and 6,063,402).

One of ordinary skill in the art can determine and prescribe the effective amount of the therapeutic composition required based on clinical protocols. In general, a suitable

daily dose of a composition of the invention will be that amount of the composition, which is the lowest dose effective to produce a therapeutic effect.

Embodiments of the invention demonstrate that administration of a mixture of a polysaccharide and a cytotoxic therapeutic may result in reduced toxicity and may further provide an enhanced therapeutic effect in comparison with the therapeutic agent in the absence of the polysaccharide. A mixture may be formed from galactomannan and any synthetic therapeutic agent so as to achieve enhanced efficacy of therapeutic effect of the agent. An example of a polysaccharide with this activity is galactomannan. Galactomannan may be obtained from a variety of natural sources such as plants and may be made synthetically by enzymatic reactions or by chemical synthesis. Examples 1 and 2 show the effects of using galactomannans derived from two separate plant sources which have been demonstrated to be effective at reducing toxicity of therapeutic agents. In particular, Example 1 describes the use of galactomannan from a second plant species *Gleditsia triacanthos* and Example 2 describes the use of galactomannan obtained from the plant species *Medicago falcata*.

Galactomannan is a polymer that may occur in a variety of size ranges. Moreover, the galactomannan may be derivatized or hydrolyzed to result in fragments of the native molecule or may be reacted to provide chemically modified forms of the native molecule. Embodiments of the invention provide a galactomannan having a molecular weight in the range of 20,000 - 600,000 D. The galactomannan may further have a size in the range of 90 - 415,000 D or 40,000 - 200,000 D. Example 1 utilizes a galactomannan with an average molecular weight of 215,000 D while Example 2 utilizes a galactomannan with an average molecular weight of 83,000 D.

The ratio of mannose to galactose may vary according to the source of the galactomannan and the isolation procedure. In embodiments of the invention, the galactomannan may have a mannose to galactose ratio of 1-3 mannose: 0.3-1.5 galactose. The ratio of mannose to galactose may be 2.6:1.5 or 2.2:0.9 or 1.13:1 or 2.2:1. In Example 1, the ratio of mannose to galactose is 2.2:1 and in Example 2, the selected ratio of mannose to galactose in the galactomannan is 1.13:1. In Example 3, the galactomannan has a mannose to galactose ratio of 2.2: 1.0.

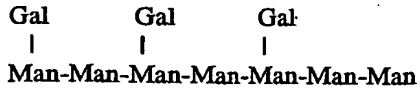
The galactomannan may be provided with the therapeutic agent in a mixture at a ratio of 0.1:1w/w to 10:1w/w with the therapeutic agent. In Example 1, the ratio of galactomannan to 5-FU is 1:1.9 and in Example 2 the ratio of galactomannan to adriamycin is 1:0.6. In Example 3, the ratio of galactomannan to 5-FU is 1.6:1. The

results shown in Examples 1 and 2 are show significant reduction in toxicity when chemotherapeutic agents are administered in the presence of galactomannan. In Example 3, the results show significant increase in efficacy observed when chemotherapeutic agents are administered in the presence of galactomannan.

These results are dramatic as shown in Example 1 where instead of a death rate of 3/5 mice with 5-FU with the surviving mice showing substantial lack of normal weight increase, the same dose administered with galactomannan results in 0/5 mice dying. All mice survive and the surviving mice have weights equivalent to control mice (treated with saline). The surviving mice appear normal in all aspects with no sign of toxicity. In Example 2, the results demonstrate the advantages of formulating a mixture of adriamycin with galactomannan. Animals treated with an LD_{50} dose of adriamycin according to standard toxicity tests result in a mortality of 3/5 mice. In contrast, when adriamycin is coadministered with adriamycin, the toxicity is reduced so that only 1/5 mice die. Moreover although there is some weight loss in the mice that survive, this weight loss is diminished.

In Example 3, a substantial reduction in tumor weight was observed in which tumor mass in control untreated mice was 2,058 mg and in mice treated with 75 mg/kg of 5-FU, tumor mass was 2,254 mg at 56 days after the initiation of treatment (the last day of the study). The same dose of 5-FU administered with galactomannan resulted in a tumor weight of 405 mg. Thirty-five days after the initiation of treatment, tumor weight was 2,450 mg (control, untreated), 990 mg (5-FU, 75 mg/kg), and 288 mg (same dose of 5-FU in combination with galactomannan). Twenty-eight days after the initiation of treatment, tumor weight was 1,296 mg (control, untreated), 527 mg (5-FU, 75 mg/kg), and 144 mg (same dose of 5-FU in combination with galactomannan).

In a preferred embodiment, the structure of galactomannans is a poly-β-1→4-mannan backbone, with the side substituents bound via α-1→6-glycoside linkages, for example:



Without being bound by any particular theory, three possible mechanisms may account for the beneficial effect of galactomannan in a mixture with a cytotoxic or chemotherapeutic drug. One involves a direct physical interaction between the drug and

galactomannan. For example, galactamannan may increase cancer cell membrane fluidity and permeability, as a result of galactose-specific interactions at the surface of the target cell. The polysaccharide can thus serve as an effective vehicle for delivery of the drug to the target. With respect to the treatment of cancer with chemotherapeutic agents, galactomannan may act to inhibit aggregation of tumor cells and their adhesion to normal cells, so that the cancer fails to metastasize. Once the polymer drug conjugate enters the tumor, which galactomannan recognizes by virtue of its structure and composition, galactomannan may release the anti-cancer drug. The toxicity of the chemotherapy drug may be reduced because the drug is inactive as long as it is bound to the polymer. Once the polymer drug conjugate enters the tumor, which galactomannan recognizes by virtue its structure and composition, galactomamman may release the anticancer drug. Another possible mode of action of galactomannan may involve its interaction with some regulatory sites in a biological system, particularly if those sites are governed by galactose-specific residues, such as galectins. Yet another possible mode of action may involve an inhibitory effect of galactomannans of a certain chemical structure (a certain Man: Gal ratio) and a certain size (molecular weight) on enzymatic systems responsible for a rapid clearance of 5-FU in the body, and therefore may potentially increase the bioavailability and prolong the mean residence time of 5-FU in the body, thus improving the therapeutic profile of 5-FU in cancer therapy.

Use of the galactomannan containing formulation can have an immediate effect of increasing the responses of patients to the chemotherapy, for example, an effect is a decrease in the dosage of the agent required for effective chemotherapy, in the presence of the formulation. It can have an immediate beneficial effect for the patient by decreasing toxicity of the drugs as here exemplified but not limited to adriamycin and 5 FU, and thereby improving a patient's quality of life.

The use of galactomannan administered in a mixture with a cytotoxic agent can be applied to a wide range of agents and is not restricted to anti-tumor or anti-cancer agents. Therapeutic areas include anti-depressants, anti-inflammatory agents, gastroenterology drugs (for treating ulcers and associated disorders), anti-psychotic drugs, anti-hyperlipidemic agents, etc. As many therapeutic agents must be administered as a chronic medicine, i.e., on a long-term basis, potential reduction in dosage and improvement in quality of life become significant factors in availability, cost of therapeutic agents, and patient compliance.

Examples of chemotherapeutic agents according to embodiments of the invention include: 1.alkylating agents such as mustargen-nitrogen mustard, cyclophosphamide (cytoxan), melphalan (alkeran), chlorambucil (leukeran), cis-platinum ("non-classical" alkylating agent), carbo-platinum ("non-classical" alkylating agent), carmustine (BCNU), thiotepa, busulfan (myleran) 2.vinca alkaloids and related substances such as vincristine, vinblastine, VP-16; 3.anthracycline antibiotics such as doxorubicin (adriamycin), actinomycin D, daunorubicin (daunomycin), bleomycin, idarubicin, mitoxantrone 4.glucocorticoids such as prednisone/prednisolone, triamcinolone (vetalog), 5.inhibitors of protein/DNA/RNA synthesis, methotrexate, 6-thioguanine, 5-fluorouracil (5-FU), cytosine arabinoside (ara-C, cytosar), L-asparaginase (Elspar), dacarbazine (DTIC), hydroxyurea (hydrea), procarbazine (matulane) and 6. miscellaneous agents such as paclitaxel.

Examples of therapeutic agents that may be administered with galactomannan to reduce their toxicity or enhance efficacy include the following: Anti-infectives including antibiotics, antivirals and vaccines, antineoplastics, cardiovascular drugs including antiarrythmics, antihypertensives etc., central nervous system drugs including analgesics, anorectics, anticonvulsants, anti-inflammatories and tranquilizers etc. OTICS, Opthalmics, gastrointestinal including anti-ulcer drugs, anticholinergic drugs etc. hormones, respiratory drugs including allergy medications, bronchodilators and decongestants, topical drugs and vitamins and minerals. Particular examples in the above categories are provided by way of illustration. Prilosec (AstraZeneca) described in U.S. Patent 4,255,431 and Prevacid (TAP) described in U.S. Patent 4,628,098; Lipitor (Pfizer) an anti-cholesterol drug described in U.S. Patent 5,273,995. The antihyper-lipidemic agent, Zocor (Merck) U.S. Patent 4,444,784; anti-depressants such as Prozac (Eli Lilly) described in U.S. Patent 4,314,081; and Zoloft (Pfizer) described in U.S. Patent 4,536,518; Paxil (SmithKline Beecham) U.S. Patents 3,923,743 and 4,007,196; 4,721,723; antipsychotic agents such as Zyprexa (Eli Lilly) hematinic agents such as Epogen (Amgen), also known as Erythropoietin, and anti-inflammatory agents such as Celebrex (Searle). The formulations and dosages are provided in the Physicians Desk Reference.

The combination of cytotoxic therapeutic agent together with galactomannan can be administered in any of the methods known in the art such as in a liquid formulation, tablet, suppository, gel, cream, transdermal or topical patch or aerosol. The formulation

may be administered to a subject by any of the routes known in the art including by oral, mucosal, inhalation, or by parenteral administration as defined above.

Use of the galactomannan containing formulation can have an immediate effect of increasing the responses of patients to the chemotherapy, for example, an effect is a decrease in the dosage of the agent required for effective chemotherapy, in the presence of the formulation. The combination of 5-FU together with galactomannan can be administered in any of the methods known in the art such as in a liquid formulation, tablet, suppository, gel, cream, transdermal or topical patch or aerosol. The formulation may be administered to a subject by any of the routes known in the art including by oral, mucosal, inhalation, or by parenteral administration as defined above.

All references cited herein are incorporated by reference. The following examples are provided by way of illustrating embodiments of the invention but are not intended to be limiting.

Examples

Example 1: Loss of Acute Toxicity of the Anti-Tumor Drug 5-FU in the Presence of Galactomannan

Acute systemic toxicity of the anti-tumor drug 5-FU in the presence and absence of galactomannan was evaluated in Albino Swiss mice.

Albino Swiss mice (Harlan, Indianapolis, IN) were used as the experimental animals for measuring toxicity of therapeutic preparations following the ICH-Guideline on the Assessment of Systemic Exposure in Toxicity Studies, March 1995. Although this study was Non-GMP, it conformed to the guidelines set forth by the following references: The current FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies; AAALAC, "Guide for the Care and Use of Laboratory Animals," National Research Council, 1996. (NIH) (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99-158 November 20, 1985), Reprinted 1996; USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR Ch.1 (1/1/95) edition, Subchapter A-Animal Welfare. ISO 10993-2, 1992. The Weight/Age range: 17.8-27.3 grams/ at least 34 days old (adult) weighed to nearest 0.1 gm. The mice were healthy, not previously used in other experimental procedures minimum 5 days under the same conditions as for the actual test. Animal room temperature: 68±5°F.

The animals were observed for clinical signs immediately after injection, and daily for the duration of the study. Observations conducted included all clinical and toxicologic signs. Animals were weighed prior to injection and at the end of the observation period. Animals surviving at the end of the study were sacrificed by carbon dioxide inhalation.

There were a total of 4 groups of 5 animals each. The groups were as follows: 1) NaCl (0.9%), 2) 5-FU (17 mg/mL), 3) galactomannan (4.73 mg/mL) 4) 5-FU (17 mg/mL)+ galactomannan (9.06 mg/mL). The diluent in all cases was 0.9% NaCl. The test article solutions and NaCl (negative control) were injected intravenously via the tail vein at a dose of 0.5 mL/mouse.

The galactomannan which in nature has a molecular weight of about 800,000 D was hydrolyzed to provide a galactomannan having an average molecular weight of 215,000 D. The galactomannan from this source has a galactose to mannan ratio of 2.2.

Galactomannan was isolated from *Gleditsia triacanthos*: (Honey locust, or Sweet-locust, or Thorny-locust) Fabaceae (family Leguminosae; Legume family, Bean family). Honey locust seeds, like those of many leguminous species, have impermeable coats and thus remain viable for long periods of time. Cleaned seeds average about 6,170/kg (2,800 lb), that is about 162 mg/seed (Vines, R.A. Trees, Shrubs, and Woody Vines of the Southwest University of Texas Press, Austin, pp. 1104 (1960). Viability can be retained for several years when seeds are stored in sealed containers at 0° to 7°C (32° to 45° F) (Bonner, F.T., Burton, J.D., and Grigsby, H.C., Gleditsia L. Honeylocust, In Seeds of Woody Plants in the United States, pp. 431-433. U.S. Department of Agriculture, Agriculture Handbook 450, Washington, DC, pp. 883 (1974)). The beans of some cultivars contain as much as 12 to 13% protein, and the pods contain up to 42% carbohydrates (Matoon, H. G., Farm Use for Tree Crops, Forest Leaves, 33, 5-7, 10-11 (1943); Stoutemyer, V.T., O'Rourke, F.L., and Steiner, W.W., Some Observations on the Vegetable Propagation of Honey Locust, J. Forestry, Vol. 42, pp. 32-36 (1944). Seeds were the primary source from which galactomannan was isolated.

Isolation, purification, and characterization of galactomannan from *Gleditsia triacanthos*

(a) Disruption of seeds.

Seeds were milled, and the obtained material (crude particles) was placed into 85% ethanol in a flask equipped with a condenser, at a ratio of 10 g of milled seeds to 100 ml of ethanol. The flask was placed into a water bath and the mixture was boiled for 45

min, to eliminate low-molecular weight carbohydrates and pigments. The settled (or filtered) material was washed with a small amount of 85% ethanol and air-dried.

(b) Water Extraction.

Approximately five volumes of water were added to the air-dried material, and the mixture was left for 6 to 10 hrs to swell. Then five more volumes of water were added, the mixture was homogenized in a blender, and then stirred continuously for 9 hours at room temperature. Some more water should be added during the homogenization and/or stirring, to make the final ratio of water and the initial dried material (w/w) equal to 40.

(c) Precipitation with Ethanol.

The mixture was centrifuged at 10,000 g for 30 min; the water extract was collected, the precipitate was washed with water under stirring and centrifuged again; and the washing-centrifugation procedure was repeated. All three water extracts were combined, the resulted volume was measured and recorded.

A 10-mL volume was taken, and mixed with a 10-mL volume of 96% ethanol under stirring. Precipitation of a galactomannan is observed. The whole mixture was placed into refrigerator (4°C) overnight, the precipitate was centrifuged, collected, and washed three times with 75% ethanol with the accompanying stirring and centrifugation. Liquid phases after each centrifugation were discarded. The final fibrous precipitate was air-dried and weighed. The yield of the galactomannan was within 20% to 26% of the weight of the initial seeds.

Since the final figure gives the yield of the galactomannan in a 10-mL volume of the extract (see above), total amount of the galactomannan in the whole volume of the extract was calculated.

The precipitation/centrifugation procedure was repeated with the whole volume of the extract (less 10-mL removed in the preceding step), except the final air-drying was not complete, and the final material should be slightly wet. That is why a separate "calibration" isolation of galactomannan was needed, namely to calculate a total amount of the polysaccharide in the whole extract for the follow-up purification.

(d) Further Purification.

The wet galactomannan was dissolved in water, aiming at 10 mg/mL concentration. In order to reach such a concentration, the galactomannan was left to swell in water at 40°-50°C, and then the mixture was agitated using a homogenizer. To the

resulting solution, a fresh Fehling reagent solution (see below) was added (at the ratio of 2-3 mL per 100 mL) under continuous stirring, to precipitate a galactomannan-Cu^{+2} complex. At the end of the precipitation procedure, the mother liquor should be clear and slightly green-blue colored. An excess of the Fehling reagent solution should be avoided, since it can dissolve the forming precipitate.

The resulting precipitate was allowed to stay for 4 hours at room temperature. After the first hour, it is recommended to take an aliquot of the mother liquor and add a few drops of the Fehling reagent solution, in order to verify a completion of the precipitation. After 4 hours, the mixture was centrifuged, the precipitate was washed with cold water (at about 10°C), and centrifuged again. The mother liquor was discarded.

To recover the galactomannan from its copper complex, the precipitate was transferred into a pre-cooled in a freezer and put on ice porcelain mortar, and a cold (10°C) 5% hydrochloric acid in 96% ethanol (v/v) was added to cover the precipitate. The precipitate was sheared with a pestle to such an extent, that the material released all the dye into the solution, and converted from gel back to a fibrous material. To facilitate the shearing process, if necessary, a little amount of solution of the acid in ethanol can be added.

Four volumes of 80% ethanol (per a volume of the precipitate) were added to the mixture in the mortar, and the resulting precipitate was isolated by centrifugation. It was washed 3 to 5 times with 80% ethanol, with a centrifugation after each washing (for a complete removal of copper salt), and air-dried for 1-2 hours.

The yield of the purified galactomannan was 13% to 18% from the weight of the initial seeds.

(e) Attenuation of the Molecular Weight.

The purified galactomannan was placed into a flask (equipped with a condenser, to be later used for boiling) and dissolved in water at concentration of 6-7 mg/mL. This can be achieved after swelling (at 45° – 50°C) and stirring of the material at this temperature. The pH of the resulting viscous solution was adjusted to 2.0-2.3, using 1N hydrochloric acid. The flask equipped with a condenser was placed onto a boiling water bath for 2½ hours.

After hydrolysis, the liquid was filtered and collected, and the precipitate discarded. The liquid, that was a solution of a partially depolymerized galactomannan (DG), was neutralized with 1N NaOH to pH of 6.0-6.5, and the DG was precipitated with

1.5 volumes of 96% ethanol under continuous stirring. The precipitate and the mother liquor were placed in a refrigerator. The next day the precipitate was centrifuged, washed with 75% ethanol, and centrifuged again. The precipitate was washed with 85% ethanol, centrifuged, washed with 96% ethanol, and centrifuged again. The resulting partially depolymerized galactomannan precipitate was dried over P_2O_5. Its molecular weight was determined (in a separate experiment, the procedure see below) as 215,000 D, and mannose/galactose ratio was 2.2. Yield was 11% to 14% from weight of the initial seeds.

(f) Preparation of Fehling Reagent Solution.

The reagent solution consists of two solutions, A and B, in equal volumes.

Solution A: Dissolve in water 34.6 g of $CuSO_4$, add a few drops of H_2SO_4, and add water to the final volume of 500 mL.

Solution B: Dissolve in water 60 g of NaOH and 173 g of $KNaC_4H_4O_6x4H_2O$, and add water to the final volume of 500 mL.

Solutions A and B are combined in equal volumes immediately before using Feling reagent solution. Solutions A and B can be safely stored for two years.

(g) Complete Acid Hydrolysis (for determination of Mannose/Galactose ratio in galactomannans).

5 mg of galactomannans were placed into a glass tube and 0.5 mL of 2N sulfuric acid was added. The tube was then fused and placed into a boiling water bath for four hours. The resulting solution was diluted with an equal volume of water, and neutralized to pH 5.5-6.0 (pH is monitored with a litmus paper) with anion exchangers Dowex-1 or Dowex-2 in their HCO_3^- form. The solution was filtered, and the liquid was evaporated (e.g., using a rotor evaporator) to dryness.

(h) Determination of Mannose/Galactose Ratio in Galactomannans.

The dried acid hydrolyzate (see above) was mixed in a small flask with 1 mL of water and 25 mg of sodium borohydride, and left for 4-5 hours at room temperature for aldehyde groups of monosaccharides to be reduced. Then 1 mL of water was added, and the mixture was neutralized to pH 5.5-6.0 adding Dowex-50 in its H^+-form. The pH was monitored using litmus paper. The liquid was filtered, collected, and completely dried.

The dry residue was mixed with 1-2 mL of methanol, agitated by shaking, and dried (in order to remove boric acid as its volatile methyl ether derivative). This step was

repeated two to three times, until the white residue of boric acid had disappeared. The flask then were placed into a vacuum desiccator for two hours, and the resulting sugar alcohols were acetylated as follows:

0.3 mL of water-free distilled pyridine and 0.3 mL of water-free distilled acetic anhydride were added into the flask with dried sugar alcohols, the flask was tightly closed using a ground glass stopper and placed into boiling water bath for 60-75 min. After it the flask is removed from the bath, carefully opened, and the reaction is stopped by addition of 1 mL of methanol. The resulting mixture of pyridine and acetic ester is evaporated at 30°- 40°C using a rotary evaporator. In order to facilitate the evaporation, 1-2 mL of methanol and 1-2 mL of heptane (in that order) should be added 2-3 times in the flask. The obtained dry residue is mixed with 0.2-0.5 mL of chloroform, and the resulting solution is injected into a gas-liquid chromatograph. As an option, chromatography columns packed with 5% XE 60 on chromatone N-AW can be used. A ratio of mannose to galactose is equal to a ratio of a relative area of their respective peaks, which are identified using pure mannose and galactose as calibration sugars.

(i) Viscosity of Galactomannan Solutions, and Molecular Weight of Galactomannan.

Relative viscosity of water solutions of galactomannan is determined using the Ostwald or Ubbelohde type viscometers, calibrated with water efflux times at 25°C. Efflux times for a series of concentrations of galactomannan in the range of 5.0 to 0.5 mg/mL are determined. Data obtained are calculated as follows:

$$\eta_{rel} = \tau/\tau^{\circ},$$

where relative viscosity is equal to the ratio of efflux times for the galactomannan solution and water (at their equal volumes), and is determined at several galactomannan concentrations. For a series of galactomannan concentrations (C, mg/mL), specific viscosity is determined for each galactomannan concentration:

$$\eta_{sp} = \eta_{rel} - 1,$$

and a graph of η_{sp}/C from C, as well as $\ln \eta_{sp}/C$ from C is plotted. Both straight lines are extrapolated to the zero concentration of galactomannan (C = 0), giving the intrinsic viscosity $[\eta]$ of the galactomannan.

Molecular weight of the galactomannan is calculated from its intrinsic viscosity as

$$[\eta] = 0.168 \times DP^{0.98},$$

where DP is degree of polymerization. Since the "molecular weight" of a single repetitive unit in galactomannan is 162, molecular weight (MW) of the galactomannan is MW = 162 x DP.

The galactomannan was found to have increased solubility in a solution containing 5-FU. The 5-FU was formulated for intravenous injection at the concentration and pH provided for in the Physician's Desk Reference.

A single dose intravenous injection of the 5-FU alone or 5-FU together with galactomannan preparations was provided via the tail vein at a dose of 0.5 mL/mouse at the doses described below in (1)-(4) and observed for clinical signs immediately after injection, and daily for the duration of the study.

There were a total of 3 groups of 5 animals each. The groups were as follows: 1) 0.9% NaCl only, 2) 5-FU only (17mg/mL) 3) galactomannan (4.73mg/mL) and 5-FU (17 mg/mL) + galactomannan (9.06 mg/mL).

The dose of 5-FU was 20% above LD_{50} i.e. 420mg/kg compared with 340mg/kg for an LD_{50}.

0.9% NaCl was used as a diluent. Animals were weighed prior to injection and at the end of the observation period. Animals surviving at the end of the study were sacrificed by carbon dioxide inhalation.

As the 5-FU was injected intravenously at the LD_{50} dose, mortality was expected in 50% of the animals. The ability of the galactomannan to reduce the toxicity of LD_{50} dose of 5-FU was measured by presence or absence of mortality in animals injected with the combination of 5-FU and galactomannan.

Table 1

Group	Animal #	Body Weight (g)			Signs of Toxicity #
		Day 0 1/9/01	Day 17 1/26/01	Weight Change	
NaCl	1	19.0	26.2	7.2	None
	2	22.1	24.8	2.7	None
	3	21.6	25.4	3.8	None
	4	18.7	25.3	6.6	None
	5	17.5	24.9	7.4	None
5-FU	6	20.4	22.1	1.7	L, P
	7	20.6	*	-	D
	8	19.4	*	-	D
	9	22.5	24.0	1.5	L, P
	10	21.2	*	-	D
GM	21	18.4	22.4	4.0	None
	22	21.7	26.3	4.6	None

	23	20.4	25.2	4.8	None
	24	22.6	27.1	4.5	None
	25	22.5	27.5	5.0	None
5-FU/GM	41	19.8	26.8	7.0	None
	42	20.8	25.9	5.1	None
	43	20.3	27.1	6.8	None
	44	18.8	24.9	6.1	None
	45	22.4	27.5	5.1	None

#Summary of clinical observations. *toxicity observed. Animals died before the end of the study. L – lethargy, P – piloerection, D – death. All of the mice were male.

The in-life portion of this acute systemic toxicity test was originally 14 days. However, the first mortality was observed on day 13. Thus the in-life duration of the study was extended to 17 days.

Animals injected with NaCl alone, or the polysaccharides alone did not show any signs of toxicity and all the animals survived to the end of the study. All the animals gained weight by the end of the study. Moreover, no signs of toxicity or mortality were observed in the animals injected with 5-FU and galactomannan where the animals gained weight similar to the controls. This result was in marked contrast to the results in mice treated with 5-FU.

Example 2: Loss of Acute Toxicity of the Anti-Tumor Drug Adriamycin in the Presence of Galactomannan.

Acute systemic toxicity of the anti-tumor drug adriamycin in the presence and absence of galactomannan was evaluated in Albino Swiss mice. Mice were bred as described in Example 1. Experimental procedures followed approved governmental guidelines as described in Example 1.

The galactomannan was derived from *Medicago falcata*. The isolated galactomannan was cleaved to obtain a preparation with an average molecular weight of 83,000 D. The ratio of galactose to mannose for this preparation was 1.13. Galactomannan was isolated from *Medicago falcata* as follows:

Isolation, Purification, and Characterization of Biologically Active Galactomannan from *Medicago falcata* (Lucerne).

Seeds were the primary source of the galactomannan that was isolated from *Medicago falcata* in this study.

(a) Disruption of seeds (as in Example 1)

(b) Benzene Treatment

The dried material was mixed with 3x volume of distilled benzene, and the mixture was periodically stirred for about 45 min. The material was filtered, washed with a small amount of distilled benzene, and air-dried.

(c) Water Extraction (as in Example 1)

(d) Precipitation with Ethanol

The mixture was centrifuged at 10,000 g for 30 min, the water extract was collected, the precipitate was washed with water under stirring and centrifuged again, and the washing-centrifugation procedure was repeated. All three water extracts were combined, and concentrated four-fold at 60°-65° C using a rotor evaporator. The resulted volume was centrifuged at 5,000 rpm for 60 min for removal of proteins, which were coagulated at the bottoms of centrifuge vessels. The resulted volume was measured and recorded.

A 5 mL-volume was taken, and mixed with a 5 mL-volume of 96% ethanol under stirring. Precipitation of a galactomannan was observed. The whole mixture was placed into refrigerator (4°C) overnight, the precipitate was centrifuged, collected, and washed three times with 75% ethanol with the accompanying stirring and centrifugation. Liquid phases after each centrifugation were discarded. The final fibrous precipitate was air-dried and weighed. The yield of the galactomannan should be within 6% to 8% of the weight of the initial seeds.

Since the final figure gives the yield of the galactomannan in a 5 mL-volume of the extract (see above), total amount of the galactomannan in the whole volume of the extract was calculated.

The precipitation/centrifugation procedure was repeated with the whole volume of the extract (less 5 mL removed in the preceding step), except the final air-drying was not complete, and the final material should be slightly wet. That is why a separate "calibration" isolation of galactomannan was needed, namely to calculate a total amount of the polysaccharide in the whole extract for the follow-up purification.

(e) Further Purification (see Example 1)

The yield of the purified galactomannan should be 6.5% from the weight of the initial seeds.

(f) Preparation of Fehling Reagent Solution as in Example 1.

(g) Preparation of Dowex resins.

Dry resins are left in water overnight for swelling, then a swollen resin is placed on glass filter and the respective water solutions are passed through. For Dowex-1 to be charged to its anionic form, 4% sodium bicarbonate solution is slowly passed through, and then the resin is washed with water until passing through water has the neutral pH (monitored with litmus paper). For Dowex-50 to be charged to its cationic (H^+) form, 3-4 volumes of 1 N hydrochloric acid is passed through, and then the resin is washed with water as described above until pH of water is neutral.

(h) Complete Acid Hydrolysis (as in Example 1).

(i) Determination of Mannose/Galactose Ratio in Galactomannans (see Example 1)

(j) Viscosity of Galactomannan Solutions, and Molecular Weight of Galactomannan (see Example 1).

The galactomannan was found to have increased solubility in a solution containing adriamycin A. The adriamycin was formulated for intravenous injection at the concentration and pH provided for in the Physician's Desk Reference.

A single dose intravenous injection of the adriamycin alone or adriamycin together with galactomannan preparations was provided via the tail vein at a dose of 0.5 mL/mouse at the doses described below in (1)-(3) and observed for clinical signs immediately after injection, and daily for the duration of the study.

There were a total of 3 groups of 5 animals each. The groups were as follows: 1) 0.9% NaCl only, 2) Adriamycin only (1.1mg/mL) and Adriamycin (1.1mg/mL) + galactomannan (7.2mg/mL). LD_{50} for Adriamycin (i.v. in mice) is 21.1 mg/kg (The Merck Index, 12th Edition, p 582).

0.9% NaCl was used as a diluent. Animals were weighed prior to injection and at the end of the observation period. Animals surviving at the end of the study were sacrificed by carbon dioxide inhalation.

As the Adriamycin was injected intravenously at the LD_{50} dose, mortality was expected in 50% of the animals. The ability of galactomannan to reduce the toxicity of LD_{50} dose of Adriamycin was measured by presence or absence of mortality in animals injected with the combination of Adriamycin and the particular polysaccharide.

Results

Animals injected with NaCl alone did not show any signs of toxicity and all the animals survived to the end of the study. All the animals gained weight by the end of the study.

Three out of 5 animals in the Adriamycin alone group (one animal each on day 1, day 4 and day 5) died before the end of the study. The surviving 2 animals lost weight by the end of the study (Table I).

One out of 5 animals injected with Adriamycin and galactomannan (one animal on day 4) died before the end of the study. Three out of four remaining animals lost weight by the end of the study. The fourth animal gained very little weight (Table I).

Observations conducted included all clinical and toxicologic signs. Adriamycin only at the LD_{50} dose caused death in 3 out of 5 mice. However, mice injected with the combination of galactomannan and a LD_{50} dose of Adriamycin resulted in the death of only one mouse demonstrating that galactomannan has the ability to decrease the toxicity of the anti-tumor drug Adriamycin.

Table 2: Effect of Galactomannan (GM) when co-administered with Adriamycin

Group	Animal #	Body Weight (g)			Signs of Toxicity #
		Day 0 02/05/01	Day 14 02/19/01	Weight Change	
NaCl	1	21.8	26.0	4.2	None
	2	17.8	30.2	12.4	None
	3	27.0	29.9	2.9	None
	4	23.6	27.5	3.9	None
	5	25.7	33.2	7.5	None
Adriamycin	6	27.3	25.2	-2.1	None
	7	22.7	19.1	-3.6	None
	8	21.0	*	-	D
	9	25.2	*	-	D
	10	21.6	*	-	D
Adriamycin/GM	16	25.3	*	-	D
	17	25.1	23.2	-1.9	None
	18	25.8	24.2	-1.6	None
	19	24.7	23.7	-1.0	None
	20	24.5	25.6	1.1	None

#Summary of clinical observations. *toxicity observed. Animals died before the end of the study. D = death. Male animals were used throughout the study

Example 3: Synergistic Effect on Tumor Reduction of the Anti-Tumor Drug 5-FU in the Presence of Galactomannan from *Gleditsia triacanthos*.

Response of subcutaneously implanted COLO 205 human colon tumor to treatment with a cytotoxic chemotherapeutic agent, 5-fluorouracil (5-FU), in the presence and absence of galactomannan was evaluated in male NCr-nu athymic nude mice.

Male NCr-nu athymic nude mice (Frederick Cancer Research and Development Center, Frederick, MD) were acclimated in the laboratory one week prior to experimentation. The animals were housed in microisolator cages, five per cage in a 12-hour light/dark cycle. The animals received filtered water and sterile rodent food *ad libitum*. The animals were observed daily and clinical signs were noted. Weight of the animals was in the range of 25-34 g at 13th day of the study, that is the first day of treatment initiation. The mice were healthy, not previously used in other experimental procedures.

Thirty to forty mg fragments of COLO 205 human colon tumor were implanted subcutaneously (s.c.) in mice near the right axillary area using a 12-gauge trocar needle and allowed to grow. Tumors were allowed to reach 75-198 mg in weight (75-198 mm^3 in size) before the start of treatment. A sufficient number of mice were implanted so that tumors in a weight range as narrow as possible were selected for the trial on the day of treatment initiation (day 13 after tumor implantation). Those animals selected with tumors in the proper size range were divided into the various treatment groups. The median tumor weights in each treatment group ranged from 94 to 117 mg.

Study duration was seventy days after tumor implantation, or fifty-six days after treatment initiation. Any animal whose tumor ulcerated or reached 4000 mg in size was sacrificed prior to study termination.

The individual animal's time to reach the evaluation size (time to reach two tumor mass doubling) was used in the calculations of the overall delay in the growth of the median tumor [(T-C)/C x 100, %] and as the endpoint in life tables analysis (stratified Kaplan-Meier estimation followed by the Mantel-Haenszel log-rank test) in order to statistically compare the growth data between groups.

The s.c. tumors were measured and the animals were weighed twice weekly starting with the first day of treatment. Tumor volume was determined by caliper measurements (mm) and using the formula for an ellipsoid sphere: LxW2/2=mm^3, where L and W refer to the larger and smaller dimensions collected at each measurement. This formula was also used to calculate tumor weight, assuming unit density (1 mm^3 = 1 mg).

The galactomannan which in nature has a molecular weight of about 800,000 D was cleaved to provide a galactomannan having an average molecular weight of 215,000 D. The galactomannan from this source has a galactose to mannan ratio of 2.2. Source of galactomannan and its isolation, purification and characterization are as described in Example 1.

Galactomannan was administered intravenously (i.v.) once every four days for a total of three injections (q4d x 3) at a dosage of 120 mg/kg/dose (except of a 60 mg/kg dosage, see below) or was co-administered as one injection with 5-FU on the same q4d x 3 treatment schedule at dosages of 120 mg/kg/dose of galactomannan and 75 mg/kg/dose of 5-FU. 5-FU alone was administered i.v. on the same q4d x 3 treatment schedule at dosages of 75 mg/kg/dose. 5-FU was formulated in saline fresh on each day of treatment at a concentration of 3.75 mg/mL, at pH 8.4-9.0 (with 1N NaOH). In the groups where galactomannan and 5-FU were co-administered, galactomannan powder was dissolved in the 5-FU solution to yield the galactomannan concentration of 6 mg/mL and 5-FU concentration of 3.75 mg/mL. Both individual compounds and their mixture were administered by exact body weight with injection volume being 0.2 mL/10 g body weight.

There were a total of seven groups of 10 animals each, s.c.-implanted with COLO 205 human colon tumor xenografts. The groups were treated on day 13 after tumor implantation on q4d x 3 schedule (except for the last group, that was treated for comparative purposes with a lower dose of galactomannan alone on q1d x 5 schedule, see Table 1) as follows:

(1) Saline (NaCl, 0.9%),

(2) 5-FU (75 mg/kg),

(3) Galactomannan (120 mg/kg),

(4) 5-FU (75 mg/kg) + Galactomannan (120 mg/kg),

(5) 5-FU (375 mg/kg),

(6) 5-FU (375 mg/kg) + Galactomannan (120 mg/kg),

(7) Galactomannan (60 mg/kg) for five consecutive days (q1d x 5).

The data (except of groups 5 and 6, see below) are shown in Table 1.

5 Table 1

	Animal response in the following five groups (10 mice in each at treatment initiation)				
	Saline (control) q4d x 3	5-FU (75 mg/kg) q4d x 3	Galactomannan (GM) (120 mg/kg) q4d x 3	5-FU (75 mg/kg) + GM (120 mg/kg) q4d x 3	GM (60 mg/kg) q1d x 5
Median days to 2x doubling of tumor weight	12.5	23.7	15.5	56.0	20.0
Animals with small tumors (20% and less) compared to median of untreated after 4-8 weeks of treatment	0	1	1	4	0
Tumor complete regression after 56 days of treatment	0	0	0	1	0
Median tumor weight (on days after treatment initiation)					
Day 0	111 mg	101 mg	100 mg	111 mg	117 mg
Day 3	189 mg	135 mg	162 mg	144 mg	162 mg
1 week	415 mg	198 mg	258 mg	209 mg	216 mg
2 weeks	527 mg	245 mg	320 mg	158 mg	319 mg
3 weeks	968 mg	352 mg	629 mg	126 mg	512 mg
4 weeks	1296 mg	527 mg	959 mg	144 mg	690 mg
5 weeks	2450 mg	990 mg	1692 mg	288 mg	1116 mg
6 weeks	1651 mg	1345 mg	1690 mg	288 mg	1421 mg
7 weeks	2432 mg	1881 mg	1764 mg	320 mg	1152 mg
8 weeks	2058 mg	2254 mg	1813 mg	405 mg	1152 mg

Animals died (on weeks after treatment initiation)					
1 week	0	1 (nonspecific)	0	0	1 (nonspecific)
2 weeks	0	2 (nonspecific)	0	4 (nonspecific)	0
3 weeks	1	0	0	0	0
4 weeks	0	0	0	1	1
5 weeks	0	1	0	0	0
6 weeks	1 (sac)	0	1 (sac)	0	0
7 weeks	1 (sac)	0	0	1 (sac)	1 (sac)
8 weeks	2 (sac)	1 (sac)	1 (sac)	0	0
Total	5	5	2	6	3
Mean survival time, days	14.2	23.7	19.2	44.2	18.1

(sac) – sacrificed animals

Control untreated tumors grew well in all mice, with a median to quadrupling of tumor weight equals to 12.5 days. There was no tumor regression after 56 days of the study, and there was practically no tumor reduction. Median tumor weight increased from 111 mg at treatment initiation (in this case with saline only) to 2000-2450 mg after 5-8 weeks. One mouse died and four more animals were sacrificed to the end of the study due to either a large tumor (>4 grams) or tumor ulceration. Mean survival time calculated using parametric models and stratified Kaplan-Meier estimation followed by the Mantel-Haenszel log-rank test was equal to 14.2 days.

5-FU administered alone at a dosage of 375 mg/kg/dose at q4d x 3 schedule was lethal, causing nine death out of ten mice within 10 days after the treatment initiation (a single LD50 dose for 5-FU in mice was reported to be 340 mg/kg, see the Physician's Desk Reference 48th Edition (1994), pp. 1925). The same treatment in a combination with galactomannan (120 mg/kg/dose) caused seven deaths within the same time period. The data are not shown in Table 1.

A dosage of 75 mg/kg/dose of 5-FU (that is, 225 mg/kg total dose over 8 days) was in excess of the maximum tolerated dosage producing three treatment-related deaths out of ten mice within two weeks. The treatment caused a delay in a median to quadrupling of tumor weight from 12.5 to 23.7 days. Again, there was no tumor regression after 56 days of the study, however, two relatively small tumors were observed that grew from 75 mg each at initiation of treatment to 126 mg and 567 mg by the end of the study. Median tumor weight increased from 101 mg at treatment initiation to 2254 mg after 56 days of the study. Three nonspecific deaths were observed within two weeks (apparently, due to toxicity), one mouse died on fifth week, and one more was sacrificed to the end of the study because of tumor ulceration. Mean survival time shifted from 14.2 days (control, untreated animals) to 23.7 days.

Galactomannan at a dosage of 120 mg/kg/dose administered alone on a q4d x 3 schedule was well tolerated without deaths or body weight loss, with a median to quadrupling of tumor weight equals to 15.5 days, that is slightly (3 days) delayed compared with untreated animals. There was no tumor regression after 56 days of the study, however, two relatively small tumors (compared to median tumor weight) were observed that grew from 100 mg and 126 mg at initiation of treatment to 270 mg and 729 mg, respectively, by the end of the study. Median tumor weight increased from 100 mg at treatment initiation to 1813 mg after 56 days of the study, that is noticeably less compared to 2000-2450 mg for untreated animals, and 2254 mg for 5-FU (75 mg/kg/dose)-treated animals. Two mice were sacrificed to the end of the study, one due to a large tumor (>4 grams), another because of tumor ulceration. Mean survival time shifted from 14.2 days (control, untreated animals) to 19.2 days.

A change of the administration schedule for galactomannan from 120 mg/kg/dose, once in four days, three injections (q4d x 3) to 60 mg/kg/dose every day, five injections (q1d x 5) caused a further delay in quadrupling of the tumor weight, from 12.5 days in control (untreated animals) to 15.5 days and 20.0 days, respectively (see Table 1). Mean survival time was close for the two schedules for galactomannan administration, namely 19.2 and 18.1 days (Table 1), however, median tumor size was significantly smaller with a more frequent administration of a lower dose of galactomannan (1813 mg and 1152 mg, respectively), and even smaller compared with that for 5-FU administration (2254 mg).

Co-administration of galactomannan (120 mg/kg/dose) and 5-FU (75 mg/kg/dose) on a q4d x 3 schedule brought a remarkable effect. It caused a significant delay in quadrupling of tumor weight, from 12.5 days for untreated animals (control) and 23.7 and

15.5 days for 5-FU alone and galactomannan alone, respectively, to 56.0 days for their combination. There was one tumor that completely disappeared by the end of the study; it went from the initial 75 mg to 126 mg on a third day after treatment initiation (that is, after the first injection) and further to 144 mg after the second and third injections, and after two weeks on the study it decreased to barely detectable one, and then completely disappeared. Two more tumors were of a relatively small size (352 and 405 mg) by the end of the study. Overall, median tumor weight increased from 111 mg at treatment initiation to only 405 mg after 56 days of the study, that is significantly less compared to 2000-2450 mg for untreated animals, and 2254 mg for 5-FU (75 mg/kg/dose)-treated animals. Toxicity, however, was still there, with four nonspecific deaths within two weeks, one death after four weeks, and one sacrificed mouse to the end of the study because of tumor ulceration. Mean survival time shifted from 14.2 days (control, untreated animals) and 23.7 days (5-FU treatment) to 44.2 days for a combination treatment.

Thus, this result was in marked contrast to the results in cancer-carrying mice treated with 5-FU alone.

What is claimed is:

1. A method for treating a cancer in a subject, comprising:

obtaining a mixture of galactomannan polysaccharide and an effective dose of a chemotherapeutic agent in a pharmaceutically acceptable formulation; and administering the formulation to the subject so as to treat the cancer.

2. A method according to claim 1, wherein the mixture contains an amount of galactomannan and the therapeutic agent in a ratio suitable for reducing a toxic effect in the subject, the toxic effect associated with administration of the chemotherapeutic agent absent galactomannan.

3. A method according to claim 1, wherein the mixture contains an amount of galactomannan and the therapeutic agent in a ratio suitable for enhancing efficacy of chemotherapeutic effect for treating the cancer.

4. A method according to claim 1, wherein the size of the galactomannan is in the range of 20,000 to 600,000 D.

5. A method according to claim 4, wherein the galactomannan has a molecular weight in the range of 90,000 to 415,000 D.

6. A method according to claim 4, wherein the galactomannan has a molecular weight in the range of 40,000-200,000 D.

7. A method according to claim 4, wherein the galactomannan has an average molecular weight of 48,000 D.

8. A method according to claim 4, wherein the galactomannan has an average molecular weight of 83,000 D.

9. A method according to claim 4, wherein the galactomannan has an average molecular weight of 215,000 D.

10. A method according to claim 1, wherein the galactomannan is a derivative of an isolate from *Gleditsia triacanthos.*

11. A method according to claim 1, wherein the galactomannan is a derivative of an isolate from *Medicago falcata.*

12. A method according to claim 1, wherein the galactomannan is a derivative of an isolate from *Cyamopsis tetragonoloba.*

13. A method according to claim 1, wherein the galactomannan is β1,4 D-galactomannan.

14. A method according to claim 4, wherein galactomannan includes a ratio of mannose to galactose in the range of 1.0-3.0.

15. A method according to claim 14, wherein galactomannan includes a ratio of 2.6 mannose to 1.5 galactose.

16. A method according to claim 14, wherein galactomannan includes a ratio of 2.2 mannose to 0.9 galactose.

17. A method according to claim 14, wherein the galactomannan includes a ratio of 1.13 mannose to 1 galactose.

18. A method according to claim 14, wherein the galactomannan includes a ratio of 2.2 mannose to 1 galactose.

19. A method according to claim 1, wherein the galactomannan and the chemotherapeutic agent are present in the mixture in a ratio of 0.1:1w/w to 10:1w/w.

20. A method according to claim 2, wherein the mixture has a reduced toxicity of greater than 50% compared with the same dose of the agent absent galactomannan.

21. A method according to claim 2, wherein the mixture has a reduced toxicity of greater than 80% compared with the same dose of the agent absent galactomannan.

22. A method according to claim 3, wherein the mixture has an enhanced efficacy of greater than 50% compared with the same dose of the agent absent galactomannan.

23. A method according to claim 3, wherein the mixture has an enhanced efficacy of greater than 80% compared with the same dose of the agent absent galactomannan.

24. A method according to claim 1, wherein the chemotherapeutic agent is adriamycin.

25. A method according to claim 1, wherein the chemotherapeutic agent is 5-FU.

26. A method according to claim 1, wherein the cancer is any of chronic leukemia, breast cancer, sarcoma, ovarian carcinoma, rectal cancer, throat cancer, melanoma, colon cancer, bladder cancer, lung cancer, mammary adenocarcinoma, gastrointestinal cancer, stomach cancer, prostate cancer, pancreatic cancer, or Kaposi's sarcoma.

27. The method according to claim 1, wherein the cancer is any of breast cancer, colon cancer, or pancreatic cancer.

28. The method according to claim 26, wherein the subject is a human subject.

29. A pharmaceutical formulation, comprising: a mixture of galactomannan polysaccharide and an effective dose for treating cancer of a chemotherapeutic agent in a pharmaceutically acceptable formulation.

30. A pharmaceutical formulation, according to claim 29, wherein the mixture contains an amount of galactomannan and the chemotherapeutic agent in a ratio suitable for reducing a toxic effect in the subject, the toxic effect resulting from administration of a cancer treating amount of chemotherapeutic agent absent galactomannan.

31. A pharmaceutical formulation according to claim 29, wherein the mixture contains an amount of galactomannan and the chemotherapeutic agent in a ratio suitable for enhancing efficacy of chemotherapeutic effect for treating the cancer.

32. A pharmaceutical formulation according to claim 29, wherein the chemotherapeutic agent is 5-FU.

33. A pharmaceutical formulation according to claim 29, wherein the chemotherapeutic agent is adriamycin.

34. A formulation according to claim 30 and 31, wherein the formulation is in a powder form.

35. A formulation according to claim 30 and 31, wherein the formulation is in a liquid form.

36. A method for treating cancer in a subject, comprising:

obtaining a mixture of galactomannan polysaccharide and an effective dose of a chemotherapeutic agent formulated so that the chemotherapeutic agent has reduced toxicity in the presence of the galactomannan, the formulation being suitable for parenteral administration to the subject; and

administering the formulation to the subject so as to treat the cancer.

37. A method for treating cancer in a subject, comprising:

obtaining an effective dose of a mixture of galactomannan polysaccharide and an effective dose of a chemotherapeutic agent formulated so that the chemotherapeutic agent has enhanced therapeutic efficacy in the presence of the

galactomannan, the formulation being suitable for parenteral administration to the subject; and

administering the formulation to the subject so as to treat the cancer.

38. A method according to claim 36 or 37, wherein the chemotherapeutic agent is adriamycin or 5 fluorouracil.

39. A method according to claim 38, wherein the enhanced therapeutic effect is a synergistic therapeutic effect.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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US CL :514/54, 883, 885; 536/123.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

STEDMANN'S DICTIONARY

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, EAST, DIALOG: BIOSIS, CURRENT BIOTECH ABS, BIOL. AND AGRIC. INDEX, EMBASE, MEDLINE, PCT FULL TEXT, IMSWORLD PATENTS, CHINESE PATENTS ABS, EUROPEAN PATENTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,773,425 A (MCANALLEY et al) 30 June 1998, see entire document.	1-3, 23, 25-32, 34-39 --- 4-9, 14-24 and 33
X --- Y	US 5,441,943 A (MCANALLEY et al) 15 August 1995, see entire document.	1-3, 23, 25-32 and 34-39 --- 4-9, 14-24 and 33
X --- Y	WO 93/08810 A1 (CARRINGTON LABORATORIES, INC.) 13 May 1993, pages 1, 15, 25, 26, 30, 84, 85, 87, 88 and 97.	1-3, 23, 25-32, 34-39 --- 4-9, 14-24 and 33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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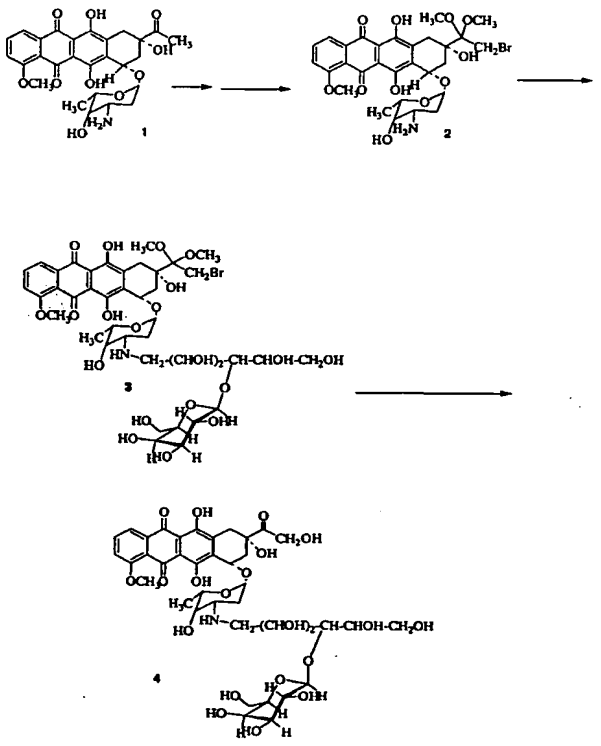
[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR REDUCING SIDE EFFECTS IN CHEMOTHERAPEUTIC TREATMENTS



(57) Abstract: A pharmaceutical compound and a process for making the compound is provided where the compound includes a therapeutic agent, a spacer and a galactose, the spacer being covalently linked to the therapeutic agent at a first site on the spacer and covalently linked to the galactose by an ether bond at a second site on the spacer to form a conjugate. The conjugate may be used to treat a subject suffering from a medical condition, so as to reduce the side effects associated with the therapeutic agent by administering an effective dose of the conjugate to the subject so that the side effects in the subject are less then they would have been with the unconjugated therapeutic agent.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Methods and Compositions for Reducing Side Effects in Chemotherapeutic Treatments

Technical Field and Background Art

The present invention relates to reducing side effects of therapeutic agents in a subject without substantial loss in efficacy where the agents would otherwise have significant side effects. This beneficial effect is achieved by coupling a galactose residue to the agent via a spacer.

Directed delivery of an agent to a target site is desirable to minimize side effects in patients and to enhance therapeutic efficacy. Side effects are the hallmark of many chemotherapeutic agents which otherwise are effective in reducing tumor size. For example, anthracycline antibiotics such as Adriamycin (also called 14-hydroxydaunamycin or doxirubicin) are effective anti-tumor agents. (Arcamone, "Doxorubicin: Anti-Cancer Antibiotics", Medicinal Chemistry Series, (1981) Vol. 17, Academic Press; C.R. Hutchinson, "The Biosynthesis of Tetracycline and Anthracycline Antibiotics," in Antibiotics IV Biosynthesis, (1981) pp. 1-11, Ed.: J.W. Corcoran, Pub.: Springer-Verlag; R.J. White, "Anthracyclines," in Biochemistry and Genetic Regulation of Commercially Important Antibiotics, (1983) p. 277-291, Ed.: L.C. Vining, Pub.: Addison Wesley).

In addition to the desired effect of destroying cancer cells, anthracycline antibiotics also damage non-cancer cells resulting in side effects for the patient. These side effects limit the dose and duration of treatment with these agents. Attempts have been made to reduce the side effects of this class of therapeutic agent. In US Patent No. 5,814,608, the daunosamine moiety of the Adriamycin was substituted with several disaccharide moieties and the modified agent tested using human tumor cell lines showing a marked reduction in cytotoxic potency for the target tumor cells (Arcamone, 1981). These *in vitro* assays did not however measure side effects which arise in a patient nor indeed did they provide information on cytotoxic effects on non-target cells. An analog of doxorubicin has been made in which a disaccharide replaces the monosaccharide daunosamine of doxorubicin. These compounds actually had increased side effects (Zunino et al., Biochemical Pharmacology (2001) Vol. 61, pp. 933-938; Gonzalez-Paz et al. European Journal of Cancer (2001) Vol. 37, pp. 431-437). It was shown that natural mono-, di- and tri-saccharide derivatives of pyrromycinone possess a progressively increased DNA binding activity with the increase in the length of the oligosaccharide chain. (DuVernay V.H.,

"Molecular Pharmacology of Anthracycline Antitumor Antibiotics", in Canver and Chemotherapy, (1981) Vol. III, pp. 233-271, Academic Press, New York.) Although an increase in DNA binding activity was shown, this does not actually mean anti-tumor potency.

Other approaches to reducing side effects have been developed which rely on directing therapeutic agents in the form of pro-drugs to their target site of action. Pro-drugs of Adriamycin were made in which the Adriamycin was linked to spacers at C14, which in turn were linked to ligands, the ligands including monosaccharides. The pro-drugs were then linked to antibodies which directed the pro-drug to target cells. The pro-drugs were hydrolyzed by enzymes which were co-administered with the pro-drugs. The intended result was liberation of the active agent at the target site only. (Leenders et al., Tetrahedron Letters (1995) Vol. 36, pp. 1701-1704; Ghosh et al., Tetrahedron Letters (2000) Vol. 41, pp. 4871-4874; Houba et al., International Journal of Cancer, (2001) Vol. 91, pp. 550-554). *In vivo* data suggested that pro-drugs were much less toxic compared to the parent agent (Adriamycin) and showed a somewhat higher tumor growth inhibition compared to the parent agent.

Summary of the Invention

In a first embodiment of the invention there is provided a pharmaceutical compound, that includes a therapeutic agent, a spacer and a galactose, the spacer being covalently linked to the therapeutic agent at a first site on the spacer and covalently linked, at a second site, to at least one galactose by an ether linkage. In examples of the embodiments, the spacer may be polyhydroxylated. The spacer may be an aldose or a ketose, and may further be a triose, tetrose, pentose, hexose or septose. The spacer may be have the chemical composition:

$-CH_2-(CHOH)_n-CH_2O-$ where $n \geq 0$ and < 20 or

$$-CH_2-(CHOH)_n-\underset{\displaystyle O-}{\overset{\displaystyle |}{C}}H-(CHR_2)_mCH_2OH$$

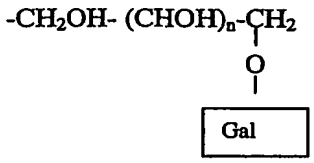
where $n \geq 0$ and < 20 and $m \geq 0$ and < 20. The presence of the spacer between a therapeutic agent and a galactose causes the galactose to be separated from the therapeutic agent by at least two carbon atoms. The covalent linkage between the spacer and the agent is formed with a reactive group on the therapeutic agent, the reactive group being selected from an amino group, an alkoxy group, a hydroxy group, a carbonyl group, a carboxylic group, a halogen and a thiol group.

In an embodiment of the invention, the therapeutic agent is Adriamycin which is covalently linked to an amide group on the daunosamine via for example an aldose or ketose

spacer. In particular embodiments of the invention, the galactose is linked to the spacer by means of a glycosidic linkage. For example, the pharmaceutical compound may include N-[β-D-galactopyranosyl-(1→ 4)-β-O-D-sorbityl]doxorubicin or N-[α-D-galactopyranosyl (1→6) 6β-O-D-sorbityl]doxorubicin.

In an embodiment of the invention, a pharmaceutical preparation, is provided that includes an effective dose of any of the pharmaceutical compounds described above and a pharmaceutically acceptable excipient.

In another embodiment of the invention, a method is provided for synthesizing a pharmaceutical compound that includes: providing (i) a therapeutic agent; and (ii) a spacer linked to a galactose conjugate; protecting reactive groups on the therapeutic agent other than the reactive site for linking to the spacer; reacting the protected therapeutic agent with the spacer linked to the galactose; and deprotecting the therapeutic agent to form the pharmaceutical compound. For example, the spacer linked to galactose has a formula:



where n=≥ 0 and ≤ 20 or 

where n=≥ 0 and ≤ 20 and m=≥ 0 and ≤ 20.

In another embodiment of the invention, a method is provided for treating a subject suffering from a medical condition, so as to reduce the side effects associated with a therapeutic agent selected for treating the condition, without substantially reducing efficacy, comprising: providing as a conjugate, the therapeutic agent covalently linked to a spacer at a first site and the spacer being covalently linked to galactose at a second site; and administering an effective dose of the conjugate to the subject so that the side effects in the subject are less then they would have been with the unconjugated therapeutic agent.

According to the above, the medical condition may include any of a proliferative condition, high cholesterol, depression, asthma, hypertension and bacterial infections. If the condition is a proliferative condition, it may include cancers such as solid tumors, an invasive tumor such as occurs in brain tumors or circulating cancer cells such as occurs in leukemia. An

example of a chemotherapeutic agent is Adriamycin the conjugate corresponding to for example, N-[β-D-galactopyranosyl-(1→ 4)-β-O-D-sorbityl]doxorubicin and N-[α-D-galactopyranosyl – (1→ 6)-β-O-D-sorbityl]doxorubicin.

Brief Description of the Drawings

The foregoing features of the invention will be more readily understood by reference to the following detailed description, taken with reference to the accompanying drawings, in which:

Fig. 1 is a synthetic pathway for N-[β-D-galactopyranosyl-(1→ 4)-β-O-D-sorbityl]doxorubicin

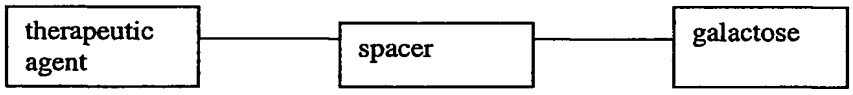
Fig. 2 is a synthetic pathway for N-[α-D-galactopyranosyl –(1→ 6)-β-O-D-sorbityl]doxorubicin.

Detailed Description of Specific Embodiments

Definitions. As used in this description and the accompanying claims, the following terms shall have the meanings indicated, unless the context otherwise requires:

Therapeutic agents that can be modified by a spacer linked to at least one galactose described in embodiments of the invention include any therapeutically active organic molecule that has a reactive group suitable for covalent attachment to the spacer so as to reduce unwanted side effects of the parent compound.

Formula 1



Galactose is a monosaccharide that belongs to the class of molecules identified as carbohydrates. Carbohydrates are attached to proteins and lipids to form glycoproteins and glycolipids. The carbohydrates play a myriad of critical roles in human metabolism. They facilitate cell adhesion and migration, and thereby help mediate the process of development. Carbohydrates aid protein function by ensuring correct protein folding, providing solubility and protease resistance, and targeting molecules both within cells and to specific cell types. Their roles in host defense include cell recognition and antigenicity.

Galactose is a hexose. It is a constituent of lactose, of plant polysaccharides (galactans) and of complex carbohydrates, such as glycoproteins, glycolipids and glycosaminoglycans. As

such, galactose is involved in many functions in an organism. Galactose affects transcription in both prokaryotes and eukaryotes. For example, yeast cells react to the presence of galactose by expressing genes necessary to utilize the sugar as a source of energy. Galactose is also implicated in transcriptional regulation in multicellular eukaryotic organisms. For example, Zinc finger activators regulate expression of genes that are induced by galactose.

We have found that when one or more galactose moieties are attached to a therapeutic agent via a spacer, side effects normally associated with the agent are reduced when the compound is administered to a subject. The spacer provides a bridge between the therapeutic agent and the galactose which facilitates orientation of the galactose with respect to the agent and is a means to avoid inappropriate interactions between the agent and galactose. Accordingly, the spacer includes 2 or more carbons in a core structure. The reactive sites with which to link the spacer to the agent and to link the spacer to galactose can be at distant ends of the spacer or may be internal to the core structure. Indeed, the spacer may have multiple reactive sites so as to facilitate the linkage of more than one galactose to the spacer in addition to the therapeutic agent. In the latter case, the galactose and the agent should nonetheless be separated on the spacer so as to avoid steric hindrance. The spacer may be polyhydroxylated although non-polyhydroxylated spacers may be used providing the reactive sites are available to enable the spacer to serve as a bridge between the therapeutic agent and galactose. In addition to the linkage groups, the spacer may further include a variety of side groups that do not interfere with the spacer function as described above.

The spacer be linked to the galactose by any appropriate means that would be recognized by one of skill in the art. For example, galactose may be linked to the spacer by means of a condensation reaction between any hydroxyl group on the galactose and a hydroxyl group on the spacer to form an ether linkage.

The spacer may be linked to the therapeutic agent by any appropriate means that would be recognized by one of skill in the art. (See for example Vladimir Torchilin "Immobilized Enzymes in Medicine" Ser. Progress in Clinical Biochemistry and Medicine (1991) Vol. 11, pp. 206, Pub.: Springer Verlag, New York, which is herein incorporated by reference). A suitable site for linking a spacer to a therapeutic agent would be through a reactive group on the agent such as for example a hydroxyl, alkoxyl, carboxylic, carbonyl, thiol, amine, halogen such as bromine, chlorine or fluorine, a sulfate or a nitrogen oxide. For example, in Figure 1, the spacer has been linked to the therapeutic agent through an amine group on the daunosamine of Adriamycin. Examples of therapeutic agents with reactive groups as described above include: Omeprazole, Simvastatin, cytosine arabinoside, cyclophosphamide (Cytoxan), melpalan

(Alkeran), chlorambucil (Leukeran), idarubicin, itoxantrone, methotrexate, 6-thioguanine, 5-fluorouracil (5-FU), cytosine arabinoside (Ara C, cytosar), L-asparaginase (Elspar), dacarbazine (DTIC), hydroxyurea (Hydrea), procarbazine (Matuline), Acetimophen, Paclitaxel, Atorvastatin, Fluoxetine, Sertraline, Albuterol, Amlodipine, Amoxicillin, Lisinophril, Clarithromycine, Cetirizine, Prevastatin, Cephalexin, Warfarin, Enalapril, Atenolol, Furosemide, Levothyroxine, Ciprofloxacin, Prednisone and Adriamycin.

A function of the spacer is to place the therapeutic agent in a suitable orientation with respect to galactose. A wide range of spacers have been described in the prior art that are designed to achieve this function and may be applicable here. (Torchilin (1991)). In an embodiment of the invention, a novel spacer in the form of an open saccharide such as an aldose or a ketose has been found to be effective with respect to galactose and a therapeutic agent.

The composition of the spacer may be as follows:

CH_2OH- $(CHOH)_n$-CH-O- where $n= \geq 0$ and <20

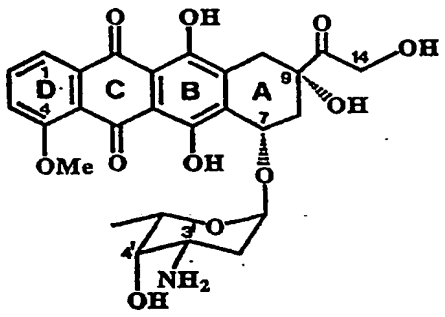
or

CH_2OH-$(CHOH)_n$- $\underset{\underset{O-}{|}}{CH}$- $(CHOH)_mCH_2OH$ where $n= \geq 0$ and <20 and $m= \geq 0$ and <20.

The galactose may be linked via an ether bond to the spacer with the galactose being in either a D form or L form. If the spacer is linked to the anomeric carbon of galactose, the linkage may be via a glycosidic bond.

In embodiments of the invention, chemotherapeutic agents which are coupled to a spacer and galactose to reduce side effects without substantially reducing efficacy can be used to treat a wide range of cancers that affect any of the tissues in the body including colon, ovary, breast, lung, pancreas, prostate, and uterus. It is envisaged that reduction in side effects of agents other than chemotherapeutic agents can be achieved through linkage of a spacer-galactose to, for example, anti-inflammatory agents, anti-psychotic agents, anti-infective agents, anti-depressants, weight reduction agents, anti-hyperlipidemic agents and anti-ulcerative agents.

"Anthracycline antibiotics" are therapeutic compounds that are widely used to treat tumors. They have a core structure consisting of an anthracycline with an attached sugar moiety on the seventh carbon, as illustrated below. Included in this definition are pharmaceutical salts as well as modifications and derivatives of the core structure such as for example, where the anthracycline is modified at the hydroxyl group on C14.



Anthracyclines with attached sugar moieties interfere with a nuclear enzyme, DNA topoisomerase II, which regulates replication, transcription and recombination of DNA. A widely used example of this class of agents is Adriamycin (14-hydroxydaunomycin, or doxirubicin) and Daunomycin. Adriamycin (doxorubicin) is a natural product, isolated from cultures of Streptomyces peutius var. caesius (U.S. patent 3,590,028). It has been synthesized from Daunomycin (J. Med. Chem., (1974) Vol. 17, pp. 659) and from 7-deoxydaunomycinone (U.S. patent 4,012,448). Anthracycline antibiotics cause severe side effects in a significant fraction of patients. For example, Adriamycin is cardiotoxic, having an LD_{50} in mice of 21.1 mg/kg (Merck Index, (1996) 12th Ed.).

Compounds of Formula 1 described herein may contain one or more asymmetric centers and can thus occur as racemates and racemic mixtures, single enantiomers, diasteromeric mixtures and individual diasteromers. Embodiments of the invention are meant to comprehend all such isomeric forms of the compounds of Formula 1. Individual tautomers as well as mixtures thereof are encompassed by compounds of Formula 1 described herein.

"Halogen" includes fluorine, chlorine, bromine and iodine.

"Subject" refers to a living animal such as a mammal including dogs, cats, pigs, cows, sheep, goats, horses, rats, and mice. The subject may be a human in need of therapy for, or susceptible to, a condition or its sequelae. An individual that is normal in all respects is not intended to be excluded in this definition.

"Proliferative disease" includes cancer, especially a tumor disease including colon, ovary, breast, lung, pancreas and uterus or leukemia; and a non-malignant proliferative disease for example, atherosclerosis, thrombosis, psoriasis, scleroderma or fibrosis.

"Patient" shall mean a human subject who has presented at a clinical setting with a particular symptom or symptoms suggesting the need for treatment.

"Saccharide" refers to any of a monosaccharide, a disaccharide, an oligosaccharide and a polysaccharide and includes substituted forms of the same.

Dosage regimens are adjusted to provide the optimum desired response, e.g., a therapeutic response. The magnitude of prophylactic or therapeutic dose of the compound of Formula 1 will, of course, vary with the nature of the severity of the condition to be treated and with the particular compound and its route of administration. It will also vary according to the age, weight and response of the individual patient.

In general, the daily dose range lie within the range of from about 0.001 mg to about 100 mg per kg body weight of a mammal, preferably 0.01 mg to about 50 mg per kg, and most preferably 0.1 to 10 mg per kg, in single or divided doses. On the other hand, it may be necessary to use dosages outside these limits in some cases.

A physician of ordinary skill in the art may determine and prescribe the effective amount of the therapeutic agent required. In general, a suitable daily dose of a compound of Formula 1 will be that amount which is the lowest dose effective to produce a therapeutic effect.

Another embodiment of the present invention provides compounds of Formula 1 and a pharmaceutically acceptable carrier. The term "preparation", as in pharmaceutical preparation, is intended to encompass a product comprising the active ingredient(s), and the inert ingredient(s) (pharmaceutically acceptable excipients) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compounds of embodiments of the invention encompass any composition made by admixing a therapeutic agent conjugate, additional active ingredient(s), and pharmaceutically acceptable excipients. "Pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, e.g., human albumin or cross-linked gelatin polypeptides, coatings, antibacterial and antifungal agents, isotonic, e.g., sodium chloride or sodium glutamate, and absorption delaying agents, and the like that are physiologically compatible. The use of such media and agents for pharmaceutically active substances is well known in the art.

Any suitable route of administration may be employed for providing a mammal, especially a human with an effective dosage of a compound of the present invention. For example, oral, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), by passage through mucosal membranes or by transdermal administration, or ocular (ophthalmic), pulmonary (aerosol inhalation), or nasal administration, can be employed. The

active compound may be delivered by continuous infusion either from an external source or from a source of the compound placed within the body.

Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments and aerosols. The most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the art of pharmacy.

Dosage forms suitable for oral administration may comprise tablets, pills, capsule, multiparticulates including: granules, beads, pellets and micro-encapsulated particles; powders, elexirs, syrups, solutions and aqueous or oily suspensions. For administration by inhalation, the compounds of the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or nebulisers. The compounds may also be delivered as powders which may be formulated and the powder composition may be inhaled with the aid of an insufflation powder inhaler device. The preferred delivery systems for inhalation are metered dose inhalation (MDI) aerosol, which may be formulated as a suspension or solution of a compound of Formula I in suitable propellants, such as fluorocarbons or hydrocarbons and dry powder inhalation (DPI) aerosol, which may be formulated as a dry powder of a compound of Formula I with or without additional excipients.

Suitable topical formulations of a compound of formula I include transdermal devices, aerosols, creams, ointments, lotions, dusting powders, and the like.

In practical use, the compounds of Formula I can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, capsules and tablets, with the solid oral preparations being preferred over the liquid preparations. Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques.

In addition to the common dosage forms set out above, the compounds of Formula I may also be administered by controlled release means and/or delivery devices such as those described in U.S. Pat. Nos.: 3,845,770; 3,916,899; 3,536,809; 3,598,123; 3,630,200 and 4,008,719.

"Efficacy" of a therapeutic agent refers to the relationship between a minimum effective dose and an extent of side effects. Efficacy of an agent is increased if a therapeutic end point can be achieved by administration of a lower dose or a shorter dosage regimen. If side effects can be decreased, a therapeutic agent can be administered on a longer dosage regimen or even chronically with greater patient compliance and improved quality of life. Further, decreased side effects of an agent enables the practitioner to increase the dosage to achieve the therapeutic endpoint sooner, or to achieve a higher therapeutic endpoint.

"Side effects" are undesirable or adverse and are associated with non-target tissue in a subject that sustains some change that is a byproduct of treatment of the target. Side effects include cytotoxicity for non-target cells and undesirable functional changes in the subject.

"Nonspecific death" is death of a treated tumor-bearing animal if its day of death was statistically less ($P \leq 0.05$) than the corresponding day of death of a tumor-bearing animal in the untreated control group.

We have exemplified embodiments of the invention by demonstrating the two different chemical syntheses used to create a therapeutic agent linked to a bifunctional spacer linked in turn to galactose.

To illustrate a general scheme of synthesis for a therapeutic agent linked to galactose via a bifunctional spacer we synthesized 14-bromodaunorubicin dimethylketal (2), obtained from Adriamycin (1) according to Povarov et al., Zh. Org. Khim., 1979, (Moscow) Vol. 15, pp.1560-1561; Olsufieva et al., Bioorgan. Khim., 1990, (Moscow) Vol. 16, pp. 856-862. The interaction of (2) with a galactose-containing disaccharide gave an Adriamycin derivative containing at the nitrogen atom of the daunosamine moiety a polyhydroxylated spacer connected with the galactose moiety via a derivative of 14-bromodaunorubicin dimethylketal. Starting from (2) and lactose (4-β-D-galactopyranosyl-D-glucopyranose) by the method of reductive alkylation with $NaBCNH_3$, N-[β-D-galactopyranosyl β 4-O-D-sorbityl]doxorubicin (4) was obtained in 8% yield, after the hydrolysis of the intermediate bromoketal (3).

Similarly starting from (2) and melibiose (6-β-D-galactopyranosyl-D-glucopyranose), N-[α-D-galactopyranosyl β 6-O-D-sorbityl]doxorubicin (6) was synthesized in 20 % yield. Both compounds (4) and (6) contain a D-galactopyranose moiety connected with the antibiotic through a hydrophilic spacer. In compound (4) D-galactose has the β-anomeric configuration, while

compound (6) has the α-configuration. In compound (4) the polyhydroxylated spacer is shorter (4 carbons) and more branched compared to compound (6) (6 carbons).

TLC and HPLC analyses show that compounds (4) and (6) contain no impurities of daunorubicin or Adriamycin. When hydrolyzed with 1N HCl (105^{0}C, 1 hour), both (4) and (6) produce adriamycinone (the aglycone) and galactose (sorbitol and daunosamine were not analyzed), as demonstrated by paper chromatography using pure adriamycinone and galactose as standards. The structures of (4) and (6) were confirmed using ^{13}C-NMR.

We have exemplified embodiments of the invention by demonstrating that when Adriamycin is modified by the addition of spacers linked to galactose, side effects are reduced as determined by reduction in non-specific death of mice while the efficacy of the agent measured in tumor bearing animals remains at least as good as the unmodified Adriamycin. (Examples 3 and 4).

Examples

Example 1: Synthesis of Galactomycin I, conjugate (6) of doxorubicin and melibiose.

To 1.3 g (2 mMol) of daunorubicin (1) in 20 mL MeOH, 10 mL dioxane, and 10 mL ethylorthoformate, 0.1 mL of Br_2 was added, and the reaction mixture was stirred for one hour at 23^{0}C. Then 0.44 g of dry K_2CO_3 were added under stirring. The precipitate was filtered off quickly, and the filtrate was evaporated in vacuum at 35^{0}C. The resulting crude 13-dimethylkethal-14-bromodaunorubicin (~ 1.5 g) (2) was dissolved in 65 mL of methanol, and 3.4 g (10 mMol) of melibiose in 30 mL of water was added. The reaction mixture was kept at 40^{0}C for four hours, then 0.275 g (4 mMol) of $NaCNBH_3$ in 0.5 mL of methanol was added, and the mixture was stirred overnight at 37^{0}C. After that 0.275 g (4 mMol) of $NaCNBH_3$ in 0.5 mL of methanol was added, and the mixture was stirred at 37^{0}C for 24 hours. This procedure was repeated twice (totally 1.1 g, or 16 mMol of $NaCNBH_3$ were added) using TLC control on silica gel (Merck 60 F254 :20 x 20 cm) in chloroform - methanol - water – formic acid (13:6:1:0.05). The resulting conjugate 5 had $R_f = 0.50$, while the starting (2) showed $R_f = 0.90$ in the same TLC system.

200 mL of water was added to the reaction mixture at room temperature, and the aqueous solution was extracted with chloroform (70 mL x 3). The organic layers were combined, and extracted with aqueous 0.25 N HBr (50 mL x 2). The dark red residue which precipitated between the layers was dissolved in 200 mL of aqueous 0.25 N HBr - methanol (1:1) mixture, and combined with the extracts of the red compound in aqueous 0.25 N HBr. The combined

acidic aqueous extracts were incubated for 6 hours at 37^0 C, then 1.5 g of HCOONa in 1 mL of water (pH ~ 4.5) was added, in order to hydrolyze 14-Br group. The reaction mixture was kept at 37^0 C for 24 hours under TLC control in the chloroform - methanol - water - formic acid (13:6:1:0.05).

The resulting crude solution was the conjugate of doxorubicine with melibiose (6) (R_f= 0.28). It was diluted with water to 500 mL, combined with approximately 100 mL of sorbent XAD-2, and stirred at room temperature for six hours until the red color of the solution disappeared. The red-colored sorbent was filtered off, washed with 500 mL of water, and compound (6) was eluted from it with the mixture of n-butanol- acetone - H_2O (13:6:1). The eluate was evaporated, the dry residue was applied onto a column with silica gel Merck 60 (0.040 – 0.063), and eluted with chloroform - methanol - water – formic acid (13:6:1:0.05). The resulting fractions, containing compound (6), were combined and evaporated in vacuum to a small volume. Pure (6) (Galactomycin I) was precipitated with isopropanol, giving 390 mg (yield of 20%, starting from daunorubicin 1) of amorphous dark red powder, with m.p. 121–123^{0}C (decomp.)

Example 2: Synthesis of Galactomycin II, conjugate (4) of doxorubicin and lactose.

Compound 4 was obtained by a similar procedure as described above, starting from 1.3 g daunorubicin (1) and lactose. Amount of 4 bromohydrate obtained was 155 mg (yield of 8%, starting from daunorubicin) with a m.p=155 – 157^{0}C (decomp.). R_f = 0.31 in chloroform - methanol - water- formic acid (13:6:1:0.05).

Example 3: Antitumor Effect of Galactomycin I and Galactomycin II compared with that of Adriamycin.

The response of subcutaneously implanted lymphocyte leukemia P-388 (Arthur D. Little Inc., Cambridge, Massachusetts) to treatment with a Galactomycin I, Galactomycin II, and Doxorubicin was evaluated in male BDF1 mice.

Mice were acclimated in the laboratory one week prior to experimentation. The animals were housed five per cage in a 12-hour light/dark cycle. The animals received filtered water and sterile rodent food *ad libitum*. The animals were observed daily and clinical signs were noted. Weight of the animals was in the range of 19-21 g at the day of treatment initiation. The mice were healthy, not previously used in other experimental procedures.

There were a total of twelve groups, 10 animals in two control groups (saline only), and six animals in each in 10 agent treatment groups. The groups were treated 24 hours after tumor implantation, as follows:

(1) Saline (NaCl, 0.9%),
(2) Saline (NaCl, 0.9%),
(3) Adriamycin (7 mg/kg),
(4) Adriamycin (14 mg/kg),
(5) Galactomycin I (7 mg/kg),
(6) Galactomycin I (14 mg/kg),
(7) Galactomycin I (20 mg/kg),
(8) Galactomycin I (40 mg/kg),
(9) Galactomycin II (7 mg/kg),
(10) Galactomycin II (14 mg/kg),
(11) Galactomycin II (40 mg/kg),
(12) Galactomycin II (80 mg/kg).

Tumor cells were implanted in a sub-cutaneous injection of one million cells per mouse and allowed to grow for 24 hours. After that, Adriamycin, Galactomycin I and Galactomycin II were administered as 0.7-10 mg/mL solutions by a single i.v. injection.

Study duration was twenty days after tumor implantation, or nineteen days after treatment initiation. Nonspecific (toxic) deaths and mean survival time, recorded in each group is shown in Table 1 below.

Both Galactomycin I and Galactomycin II are significantly less toxic compared to Adriamycin. A dose of 14 mg/kg of Adriamycin resulted in two nonspecific (toxic) deaths out of six animals, and two animals lived longer than the duration of the study (20 days). However, doses as high as 40 mg/kg (for Galactomycin I) and 80 mg/kg (for Galactomycin II) resulted in only one nonspecific death each, and in each of these cases two animals also lived longer than 20 days.

Weight loss of animals also shows that both Galactomycins I and II are less toxic compared to Adriamycin. In all the three groups, described above (Adriamycin 14 mg/kg, Galactomycin I 40 mg/kg, and Galactomycin II 80 mg/kg), when two animals in each group lived longer than the duration of the study, an average weight loss of an animal was 1.8 g (Adriamycin), 0.9 g (Galactomycin I) and 1.0 g (Galactomycin II). That is, practically the same

result in terms of survival time was reached with less side effects with Galactomycin I and II compared with Adriamycin.

Table 1

Animal group		Nonspecific deaths per group	Average weight gain/loss from day 1 to day 7 (g)	Average survival time, days	
				Including nonspecific deaths	Excluding nonspecific deaths
Agent	Dose, mg/kg				
Control (saline)	--	0	+5.2	9.7	9.7
Control (saline)	--	0	+5.2	9.1	9.1
Adriamycin	7	0	-0.5	15.5	15.5
	14	2	-1.8	>15	>19
Galactomycin I	7	0	+1.7	11.7	11.7
	14	0	+2.3	12.2	12.2
	20	0	-0.8	16.3	16.3
	40	1	-0.9	>16	>18
Galactomycin II	7	0	+4.3	11.7	11.7
	14	0	+0.8	12.0	12.0
	40	0	-1.1	15.0	15.0
	80	1	-1.0	>16	>18

Example 4: Reduction in Side Effects for Galactomycin I and Galactomycin II compared with that of Adriamycin.

Male BDF1 mice were used as the experimental animals for measuring side effects of therapeutic preparations. Mice were acclimated in the laboratory one week prior to experimentation. The animals were housed five per cage in a 12-hour light/dark cycle. The animals received filtered water and sterile rodent food *ad libitum*. The animals were observed daily and clinical signs were noted. Weight of the animals was in the range of 19-21 g at the day of treatment initiation. The mice were healthy, not previously used in other experimental procedures.

A single dose intravenous injection of Adriamycin, Galactomycin I or Galactomycin II was provided via the tail vein at the doses listed in Table 2, and the animals were observed for clinical signs immediately after injection, and daily for the duration of the study (20 days).

There were a total of 18 groups of six animals each, as shown in Table 2.

Animals injected with NaCl alone did not show any signs of side effects and all the animals survived to the end of the study.

Three out of six animals in the Adriamycin (21 mg/kg) group died before the end of the study. This was expected, since LD_{50} for Adriamycin (i.v in mice) is 21.1 mg/kg (The Merck Index, 12th Edition, p 582).

Table 2

Animal group (six animals each)		Deaths per group	Life span after treatment, days (except survived animals)
Agent	Dose, mg/kg		
Control (saline)	--	0	--
Doxorubicin	21	3	0, 1, 2
Galactomycin I	10	0	--
	15	0	--
	20	0	--
	25	0	--
	30	0	--
	40	1	2
	80	6	1, 1, 2, 2, 2, 2
	100	6	1, 1, 1, 2, 2, 2
Galactomycin II	15	0	--
	20	0	--
	25	0	--
	30	0	--
	40	0	--
	50	0	--
	80	1	2
	100	3	1, 1, 2

As was observed in Example 3, both Galactomycin I and Galactomycin II are significantly less toxic compared to Adriamycin. Data described in this Example confirm that the LD_{50} value for Adriamycin for a single injection in mice is close to 21 mg/kg. Estimated values for the LD_{50} for Galactomycin I and Galactomycin II are about 50-60 mg/kg and 100 mg/kg, respectively.

The above data illustrates how a chemical attachment of D-galactose residue to the 3'-amino group of Adriamycin via a linker (4 to 6 carbons in length) reduces side effects associated with Adriamycin 3 to 5 times, without changing efficacy of its therapeutic action for treating a particular form of cancer.

All reference cited herein are incorporated by reference.

What is claimed is:

1. A pharmaceutical compound, comprising: a therapeutic agent, a spacer and a galactose, the spacer being covalently linked to the therapeutic agent at a first site on the spacer and at a second site on the spacer, being covalently linked via an ether linkage with the galactose.

2. A pharmaceutical compound according to claim 1, wherein the spacer is polyhydroxylated.

3. A pharmaceutical compound according to claim 2, wherein the spacer is a selected from an aldose and a ketose.

4. A pharmaceutical compound according to claim 1, further comprising the agent linked to $-CH_2-(CHOH)_n-CH_2-O-$ (Galactose) where $n \geq 0$ and < 20.

5. A pharmaceutical compound according to claim 1, further comprising the agent linked to

$$-CH_2-(CHOH)_n-\underset{\displaystyle O-\text{(Galactose)}}{\underset{|}{CH}}-(CHR_2)_mCH_2OH$$

where $n \geq 0$ and < 20 and $m \geq 0$ and < 20

6. A pharmaceutical compound according to claim 3, wherein the spacer is an open chain saccharide selected from a triose, a tetrose, a pentose, a hexose and a septose.

7. A pharmaceutical compound according to claim 1, wherein the first site is separated from the second site by at least two carbon atoms.

8. A pharmaceutical compound according to claim 3, wherein the spacer is an open chain hexose.

9. A pharmaceutical compound according to claim 1, wherein a covalent linkage is formed with a reactive group on the therapeutic agent, the reactive group being selected from an amino group, a alkoxy group, a hydroxy group, a carbonyl group, a carboxylic group, a halogen and a thiol group.

10. A pharmaceutical compound according to claim 1, wherein the therapeutic agent is Adriamycin.

11. A pharmaceutical compound according to claim 10, wherein the spacer is covalently linked to an amine group on the daunosamine.

12. A pharmaceutical compound according to claim 8, wherein the galactose is linked to the spacer by means of a glycosidic linkage

13. A pharmaceutical compound according to claim 1, further comprising N-[β-D-galactopyranosyl-(1→ 4)-β-O-D-sorbityl]doxorubicin.

14. A pharmaceutical compound according to claim 1, further comprising N-[α-D-

galactopyranosyl -(1→ 6)-β-O-D-sorbityl]doxorubicin.

15. A pharmaceutical preparation, comprising: an effective dose of a compound according to claim 1 and a pharmaceutically acceptable excipient.

16. A method for synthesizing a pharmaceutical compound, comprising:

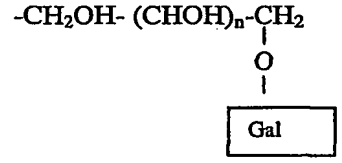
(a) providing (i) a therapeutic agent; and (ii) a spacer linked to galactose;

(b) protecting reactive groups on the therapeutic agent other than at a reactive site for linking to the spacer;

(c) reacting the protected therapeutic agent with the spacer linked to galactose; and

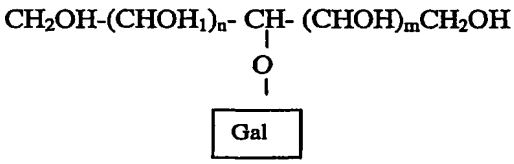
(d) deprotecting the therapeutic agent to form the pharmaceutical compound.

17. A method according to claim 16, wherein the spacer linked to galactose has a formula:



where n=≥ 0 and ≤ 20.

18. A method according to claim 16, wherein the spacer linked to galactose has a formula:



where n=≥ 0 and ≤ 20 and m=≥ 0 and ≤ 20

19. A method according to claim 16, wherein the spacer is an aldose or ketose.

20. A method according to claim 19, wherein the spacer is a hexose.

21. A method of treating a chronic disease in a subject, comprising: administering to a subject, an effective dose of a pharmaceutical compound linked via a spacer to a galactose.

22. A method of treating a subject suffering from a medical condition, so as to reduce side effects associated with a therapeutic agent selected for treating the condition without substantially reducing efficacy of the agent, comprising:

(a) providing as a conjugate, the therapeutic agent covalently linked to a spacer at a first site and the spacer being covalently linked to galactose at a second site; and

(b) administering an effective dose of the conjugate to the subject so that the side effects in the subject are less then they would have been with the unconjugated therapeutic agent.

23. A method according to claim 22, wherein the medical condition is selected from a proliferative disease, high cholesterol, depression, asthma, hypertension and bacterial infections.

24. A method according to claim 22, wherein the proliferative disease is a tumor.

25. A method according to claim 22, wherein the proliferative disease is lymphocytic leukemia.

26. A method according to claim 22, wherein the therapeutic agent is Adriamycin.

27. A method according to claim 22, wherein the spacer is selected from a ketose and an aldose.

28. A method according to claim 22, wherein the galactose is linked via an ether linkage to the galactose.

29. A method according to claim 22, wherein the spacer is selected from

(agent) -CH_2- $(CHOH)_n$-CH2-O- (Galactose) where n=$\geq$ 0 and < 20, or

(agent) -CH_2-$(CHOH)_n$- CH- $(CHR_2)_m CH_2OH$
　　　　　　　　　　　|
　　　　　　　　　　　O- (Galactose)

where n=$\geq$ 0 and < 20 and m=$\geq$ 0 and < 20.

30. A method according to claim 22, wherein the conjugate is selected from N-[β-D-galactopyranosyl-(1→ 4)-β-O-D-sorbityl]doxorubicin and N-[α-D-galactopyranosyl –(1→ 6)-β-O-D-sorbityl]doxorubicin.

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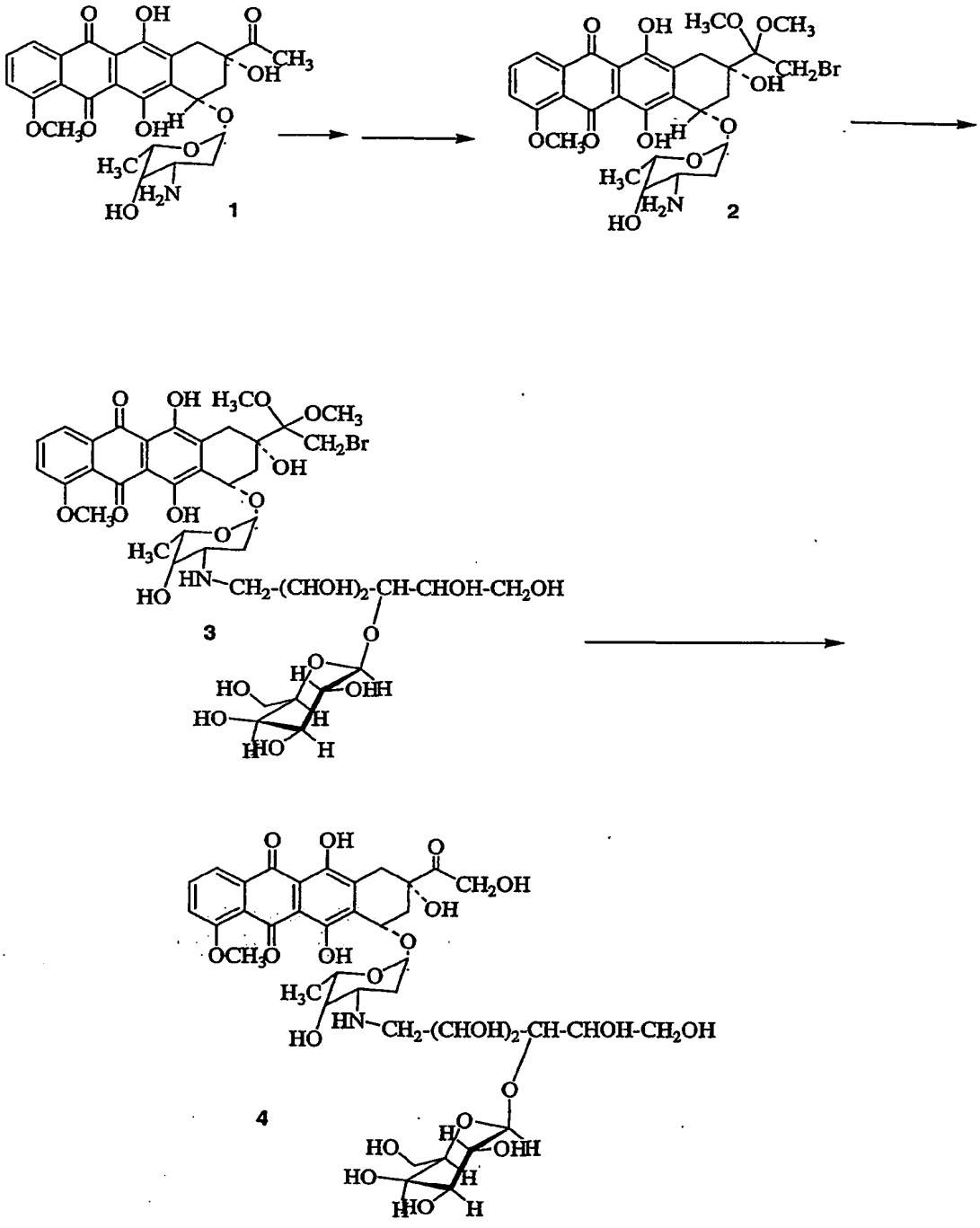
Figure 1



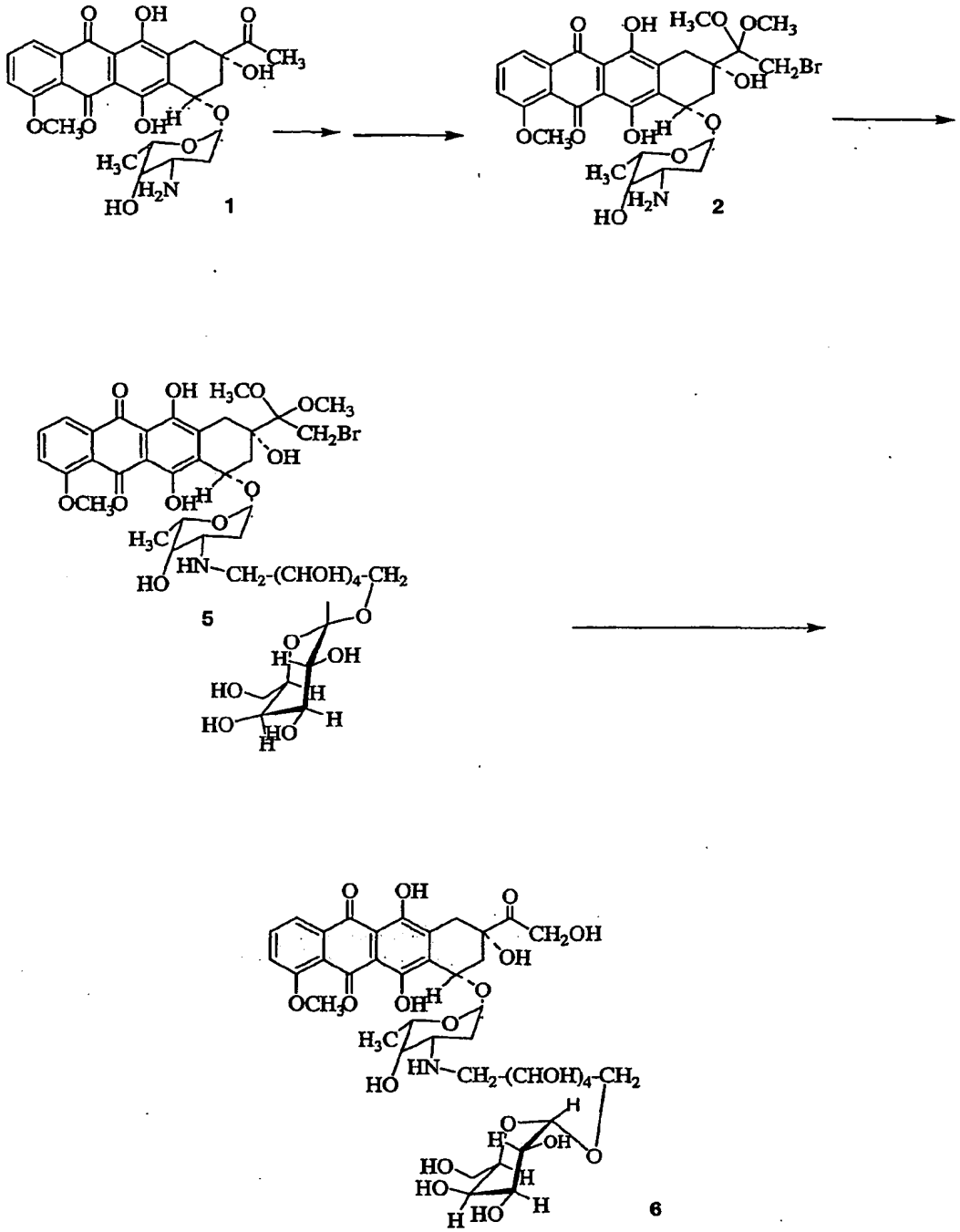


Figure 2

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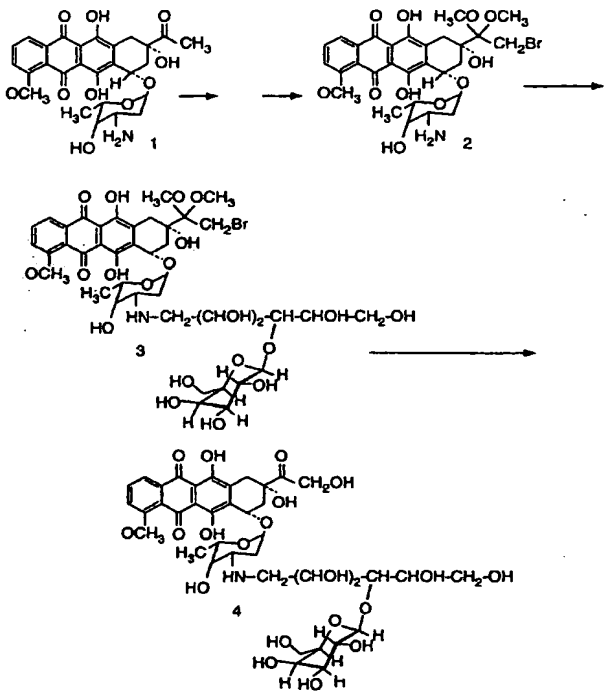
[Continued on next page]

(54) Title: COMPOSITIONS FOR REDUCING SIDE EFFECTS IN CHEMOTHERAPEUTIC TREATMENTS



(57) Abstract: A pharmaceutical compound and a process for making the compound is provided where the compound includes a therapeutic agent, a spacer and a galactose, the spacer being covalently linked to the therapeutic agent at a first site on the spacer and covalently linked to the galactose by an ether bond at a second site on the spacer to form a conjugate. The conjugate may be used to treat a subject suffering from a medical condition, so as to reduce the side effects associated with the therapeutic agent by administering an effective dose of the conjugate to the subject so that the side effects in the subject are less then they would have been with the unconjugated therapeutic agent.

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PCT/US 01/29754

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EMBASE, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 595 133 A (BEHRINGWERKE AG ;HOECHST SA LAB (FR)) 4 May 1994 (1994-05-04) claims ----	1-30
X	DE 195 24 515 A (DEUTSCHES KREBSFORSCH) 9 January 1997 (1997-01-09) claims 1,4,12-15 ----	1-30
X	PALOMINO E: "CARBOHYDRATE HANDLES AS NATURAL RESOURCES IN DRUG DELIVERY" ADVANCED DRUG DELIVERY REVIEWS, AMSTERDAM, NL, vol. 13, 1994, pages 311-323, XP000775555 ISSN: 0169-409X page 317, paragraph 4 -page 320, paragraph 3 ----	1-30
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

In onal Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 19243 A (CO ENZYME TECHNOLOGY LTD) 27 June 1996 (1996-06-27) claims 1,2,4,7,9	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/29754

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 21-30 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In tional Application No
PCT/US 01/29754

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